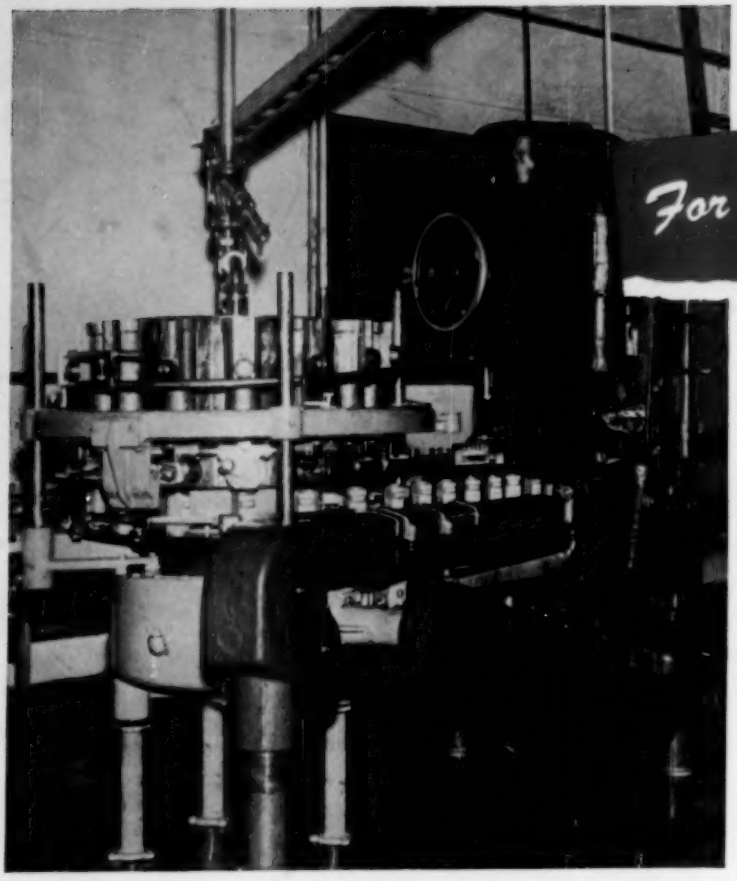
FOOD TECHNOLOGY

PUBLISHED BY THE INSTITUTE OF FOOD TECHNOLOGISTS

Volume 5, Number 8

August, 1951

UNIVERSITY OF MICHIGAN LIBRARIES



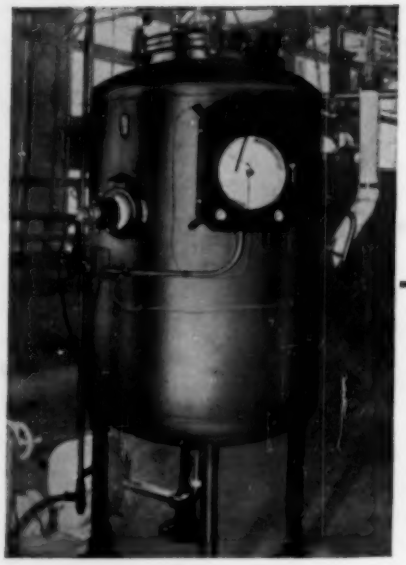
For your next citrus pack . . .

FAST PROCESSING AT LOW COST with Pfaudler Equipment

With the competition in citrus packing so keen, you will want to take advantage of these money-saving features of Pfaudler equipment.

Many packers of frozen and concentrated citrus juices have solved their oxidation problem with Pfaudler deaerators in combination with Pfaudler High-Speed Rotary Piston Fillers. To assure top quality and flavor, the deaerator removes all air just before the product is canned. With a constant vacuum maintained throughout the unit, the product is quickly, cleanly and accurately packaged by the Pfaudler Filler.

In addition, Pfaudler engineers are prepared to help you set up an efficient, economical vacuum processing unit. Use the coupon to get all the facts.



PFAUDLER ROTARY PISTON FILLERS are specially built for accurate, high-speed operation—100 to 600 containers per minute, depending on the product. An instantaneous volume adjustment controls fill within 1/10 of an ounce. Will handle containers up to 5 qt. size. The Vacuum Foods installation shown here is used for filling "Minute Maid" frozen orange juice.

PFAUDLER STAINLESS STEEL DEAERATORS can handle from 2 to 45 gallons of fruit juice per minute. To prevent flooding, an automatic liquid level control is provided. This is balanced with a vacuum differential controller. The unit shown is used by Nedicks for preventing oxidation in their famous orange drink.

Pfaudler

THE PFAUDLER CO., ROCHESTER 3, NEW YORK

Engineers and Fabricators of Food Processing Equipment

GLASS-LINED STEEL
Hastelloy—Aluminum
Tantalum—Carbon Steel
Solid or Clad Stainless Steel
Nickel Inconel Monel

THE PFAUDLER CO., Dept. FT-8, Rochester 3, N.Y.

Please send me full details on:

☐ Pfaudler Deaerators
☐ Pfaudler Piston Rotary Fillers

Name_____

Company_____

Title_____

Address_____

City_____ Zone____ State_____

New Food Products Needed by the Armed Forces[a,b]

HOWARD D. LIGHTBODY
QM Food and Container Institute, Chicago, Ill.

(Manuscript received June 9, 1951)

This title permits a very wide latitude of presentation. It appears to be a rule in military organizations to give instruction on the basis of defining objective to be attained but not to limit action by attempts to describe how the mission is to be accomplished. I am taking the liberty then to state objectives as set up by definition of military requirements. This approach places me in the very favorable and unaccustomed position of being able to describe needs without concern as to how the needs may be met. Some of these objectives may appear to you to be very unrealistic when considered within the confines of present technical knowledge. This may be, in part, due to the grouping of subsistence items in the same category as other Army materiel in setting up the requirements. For example, we are told to develop subsistence items which will withstand storage fluctuating temperatures ranging from —62° to 71° C. (—80 to 160° F.) for two years, or for one year at constant temperature of 37.8° C. (100° F.) and relative humidity of 90%, and which will remain functional through temperatures from —53.9° to 51.7° C. (—65 to 125° F.).

These may be readily attainable goals for most supply items, but quite impracticable for some food items. Ascorbic acid and thiamin losses can be expected to be severe at the upper temperature limits. Physical properties may be changed irreversibly. Off-flavors and off-colors may appear. Yet, we are not inclined to protest. Rather, it seems best to let the requirements stand as ideals to be approached and, in some instances, attainable in the distant future. Practically, we are content to define storage requirements generally as retention of quality for six months at 100° F. and accept less rigorous requirements in some instances. For example, a concession is made in the canned margarine requirement, namely, six months at 32.2° C. (90° F.), but which will not oil off at this temperature. We are asking for cake mixes that are stable for 12 months at 37.8° C. (100° F.) but will be content—for the present —with mixes that will be stable for six months at 37.8° C. (100° F.).

Perhaps it is worthwhile to take a closer look at the military use of the term "new foods"—it is rather loose. We are prone to classify an item modified only by increasing the size of meat chunks—as a concession to acceptability—a new item. Our concern is improvement and adaptation of products for military use, and we believe the loose usage of the term is justifiable. Occasionally, there is need for items that can properly be termed new. An example of this is the Soup and Gravy Base. During January and February 1951, boneless beef accounted for approximately 71% of the total beef procurement. It appears that the replacement will soon be complete where long distance shipping is involved. This is in marked contrast to the former practice of distributing carcass beef. The change deprived the military cooks of bones and trimmings used in preparing stock which was required by 70 Cook Book recipes. A substitute was required. Joint efforts of Industry and the Institute resolved the problem by devising a blend of beef extract, fat, seasonings, pigments, and protein hydrolysates. The result: a new item. Another example can be found in a powdered imitation vinegar base which is still in the development mill. Shipping and packaging costs of vinegar outstrip the costs for vinegar itself. Losses through container breakage make the availability of the item at the point of destination problematical. A product appears on the horizon. However, packaging problems, namely, a satisfactory non-breakable material, to contain it within reasonable confines are not yet resolved.

Truly, new items are in general, of questionable value. The GI is unlikely to accept them unless they can be served to resemble something to which he is accustomed. They are likely to be outside his established food habits, and although they may have a novelty appeal, they lack endurance and may be quickly rejected. Then too, strictly new products are likely to be specialty products of limited production in the measurable future. Numerous unsolicited samples of purportedly new food items are submitted to the Institute and to Food Service each month. The military usefulness of the items are estimated, sometimes involving considerable expenditure of effort. However, the yield is very low, and it is infrequent that useful advances result. On the other hand, it is difficult to close the door on purportedly new ideas lest something of real merit escape us.

There is room for a series of new items for use in emergency feeding, under conditions where men as individuals or small groups are separated from sources of supply for limited times. These conditions include isolation in life rafts, and on all types of terrain over which airplanes move. For logistic reasons, an all-purpose ration is much to be desired. The time of dependence on the ration may be many days. For this reason, nutritional adequacy is desired. However, since caloric intake is limited, nutritional balance more accurately states the requirement. The water supply may be very limited under some conditions of use. Items included must not be thirst-provoking, though body salt requirements must be met. The components must provide maximum quantities of water as an end product of metabolism without leading to intermediates and end products requiring extra water for excretion, as for

[a] Presented at the Eleventh Annual Meeting of the IFT, New York, N. Y., June 20, 1951.

[b] This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 348 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

example, the acetone bodies and urea. They must individually supply protein of best biological value and in such quantities as to permit body utilization for purposes other than energy sources. In this relation, the extension of our basic information is very desirable. Physiology supplies much information on water metabolism and water balance though efforts are being made to extend it. The psychology of thirst is a wide-open field. High caloric density and minimum weight and space demands are musts. The items included in the ration must have taste appeal and afford some variety. Items that meet these requirements do not fall within the presently accepted food categories—perhaps they more nearly approach confections. Some may be closely related to cereals, others to dairy products. Therefore, new foods must be devised, and in this instance they probably can be legitimately classified as *new* under a more rigorous definition of the word. To attain wide acceptance they must not deviate very far from items to which the men are accustomed.

In our jargon, "new foods" generally mean items that —compared to existing items—better meet the military characteristics. These, again in our jargon, are usually stated in terms of improved stability, taste appeal, nutritional adequacy, and utility. Experience as well as surveys of food preference show that it is better that the advances be on improvements of products that already are established in the American dietary regimen. The first three requirements are self-explanatory. Utility may be defined as usefulness under anticipated conditions of transportation, storage, and consumption, and for uses for which the ration is assembled and packaged.

The major research and development efforts directed toward solutions of Armed Forces feeding problems are related to operational rations, ration supplements, and food packets. Such assemblies are designed for use where refrigeration equipment is lacking and, therefore, are made up of packaged non-perishable items. Utility considerations require attention to space and weight. This brings us to dehydrated foods. There is no limit to the utilization of dehydrated foods except those imposed by reconstitution properties and taste acceptance, stability and nutritional adequacy. In practice the interests lie with beverages, fruits, vegetables, soups, meats, and dairy products.

Product improvements have led to marked recent advances in the know-how of preparing soluble coffees. The problem is held to be no longer urgent, though better aroma and flavor and more adequate specification description to assure quality of the product purchased is very desirable. An adequate soluble tea has been developed through cooperation with industry for use under conditions which permit availability of warm but not hot water for preparing the beverage. This is a new product. A dehydrated meat bar has been developed which is performing satisfactorily in special rations. This is sufficiently new in character that we trust that the stigma of pemmican is avoided. An extension in variety of dehydrated meat bars is desirable. New basic information regarding "tankage" odor, what it is and how its development can be prevented, would almost certainly lead to improvements.

Subsistence experiences from Korea are now coming back to us in reasonably good form. Some reasons for elation and some for disappointment are appearing. One of the major needs—perhaps the all important one—is for meat items that are palatable when cold—very cold. Packaged meat dishes such as stews, hashes, and meat with gravy served hot, for example, in individual or small group feeding under conditions that permit heating—have been found to be very acceptable. Supplied in combat rations in low temperature areas, where heating before serving is not possible, they are rejected, most often because of excess of free fat. Improved meat dishes and/or fish preparations that are palatable when cold are needed.

The major urgency among dairy products is for improved whole milk. This item has been on the urgent list for a long time, but there is evidence of real progress and perhaps ultimately a solution will be found. Presently available products fail to simulate fresh whole milk because of limited dispersibility and off-flavors. Failure to disperse properly is especially striking when reconstitution is attempted without mechanical equipment, a condition which prevails when individual field use is attempted. A product which will disperse rapidly and completely with finger stirring and without chalky deposition and off-flavors is needed. Here, perhaps more than in most commodities, product advances appear to be dependent on progress in the development of background information relative to the chemical and physical nature of the changes in milk components which take place during processing and storage and which result in lowered dispersion properties and off-flavors.

Turning to dehydrated vegetables, we encounter an acute problem of acceptability, probably in part traceable to food habits, individual idiosyncrasies of likes and dislikes, and to bad reputations gained in the last war. With some, as for example white potatoes and onions, acceptance of the products as now prepared and used is nearly universal. With others, such as beets, a very different situation exists, and it may even be questionable that frequent appearance of fresh or canned products on the menus designed for post, camp, and station feeding is warranted. It is hoped that an answer will be obtained through the preference surveys now in progress. On some items not now available we feel reasonably secure regarding acceptance provided satisfactory products can be made available. Among these is dehydrated corn. Drying corn for winter use was a procedure practiced by many of our pioneering forefathers (or more accurately, perhaps, our foremothers). Either their storage facilities were superior to those of the Armed Forces, or their tastes not so discriminating. If taste deterioration can be prevented and a rapidly reconstituting product devised, a new food item for overseas use would be available. Sulfiting is beneficial but the blandness of the product places a very narrow concentration range on the sulfite carried through. Maximum moisture content compatible with stability and subsequent reabsorption needs study. Here, as in many other items, non-enzymic browning is a problem and further basic information regarding the mechanism and control would accelerate progress. Dehydrated peas and green beans are wide open for study. These two

vegetables enjoy considerable favor and the acceptance of good dehydrated products seems likely as technical experience is extended.

The various off-flavors developing during storage of dehydrated carrots and sweet potatoes limit the attractiveness for concentrated efforts to improve these products. Serving them in the form to which the men are accustomed to seeing them on the tables seems almost imperative, but propounds a difficult problem. Cabbage seems close to the borderline regarding place on the menu and likelihood of success. It seems to be much higher on the required list of the British—a further illustration of differences in food habits. Acceptance of dehydrated tomatoes appears to be remote. Perhaps solution of some of the more generally applicable problems of dehydration may lend more assurance of success and warrant some concentration of effort.

The situation relative to dehydrated fruits is not unlike that of the vegetables. Some, such as apples, do very well in the present state of development, but others, as apricots, will not meet the long storage requirements for overseas use though they do show promise. There are still others—for example, the berries—where the likelihood of developing satisfactory products seems very remote indeed. Success in improving reconstitution properties and in extending storage life would constitute significant advances. Partial success in stabilizing flavors and making them available for extension of varieties of food bars for emergency feeding would be very welcome.

At present, for intake of the more heat- and oxidation-labile vitamins in operational rations, reliance is placed upon fortification of selected items that afford best protection. Soluble coffee serves as a carrier of added ascorbic acid. Some GI's do not drink coffee and because of this do consume a diet inadequate in vitamin C when subsisting on packaged items. Wider distribution of the vitamins in the items becomes a must. Jams and preserves seem likely carriers but two weaknesses appear. One of these is instability of the vitamin in the medium, the other the color instability of the products. Perhaps the two problems are closely related and the solution of one will lead to the solution of the other.

What I have given you may be considered as examples. For a more complete survey of military needs I would refer you to the booklet published by the Associates, Food and Container Institute, titled, "Food and Container Problems of the Armed Forces," specifically to the third edition which appeared 1 February 1950, for a more comprehensive statement of Armed Forces' needs for "new" foods.

Commissary Research for the U. S. Navy[a]

L. ALLEN LEVENSON

U. S. Naval Supply Research and Development Facility, Naval Supply Depot, Bayonne, N. J.

(Manuscript received May 10, 1951)

A research laboratory has been established at the Naval Supply Depot, Bayonne, New Jersey to deal with the problems of subsistence which are peculiar to the U. S. Navy. Methods of research on special foods, specialized food preparation, and food service equipment are discussed.

With the memory of feeding problems of the past war fresh in the mind, the Navy Department established its Commissary Research Division in March of 1946 to investigate problems peculiar to Naval subsistence, particularly as related to shipboard feeding. The Commissary Research Division is under the management control of the Bureau of Supplies and Accounts, and is one component of its Naval Supply Research and Development Facility under the Command of Rear Admiral Charles L. Austin, and is located at the Naval Supply Depot at Bayonne, N. J.

With food and equipment problems being investigated by laboratories of the Army, Air Force, and Navy, the Panel on Foods of the Research and Development Board, which is composed of prominent Food Technologists, determines what problems shall be attacked, and furthermore, by which of the three services the problem shall be handled. Thus, duplication of effort is obviated. In general, the Commissary Research Division has been assigned problems that revolve about pecularities of Naval subsistence.

Now what are these peculiarities of Naval subsistence? Every Naval ship, no matter its size, finds its feeding problem complicated by these limitations: (1), the space available, space for food stowage and the food preparation equipment; (2), electricity available, electricity for energizing the equipment for refrigeration and food preparation; (3), weight of provisions and equipment; (4) personnel, an insufficient number in the galley, many of whom have had little experience; and (5), inadequate food preparation facilities, inadequate in equipment, both types and amounts, and area.

Broadly speaking, then, these limitations encompass two general areas or fields that hold the interest of the Commissary Research Division: the foods themselves, and the galley, including layout and equipment.

Towards solving the foods problem, we have found ourselves investigating dehydrates and concentrates because of their space and weight saving characteristics, prefabricated meats and precooked frozen foods because of their simplicity of preparation (both as to method and time involved), and premixes. Each food item deemed possibly valuble for feeding aboard ship is first given tests at Bayonne. Our work with foods of these types embraces a study of flavor acceptability, time saving in preparation, space, weight, and electricity saving features plus determining the best method of preparation in the galley aboard ship. For these evaluations, a test galley is maintained at Bayonne which is equipped with preparation equipment normally found aboard ship.

[a] Presented at the Eleventh Annual Meeting of the IFT, New York, N. Y., June 19, 1951.

This test galley is staffed by a technologist directing naval cooks whose opinions are sought and highly respected on the theory that the most wonderful idea is good only to the extent that it is acceptable to the personnel who will have to live intimately with its application. Final testing for acceptability at Bayonne is at the enlisted men's mess hall which serves over 500 men.

Assuming that the food product in question is favorably received at Bayonne, the product is then given the "acid" test under actual operating conditions aboard ship. The tests are of the same type as just outlined, and serve to confirm or repudiate "desk" evaluations made under the more ideal circumstances existing on shore at the Research and Development Facility at Bayonne. Thus, a more accurate picture is obtained as to whether, for instance, the food actually saves space under shipboard storage conditions, whether less time is necessary in preparation as compared to the comparable conventional item, whether there is an electricity savings, whether the Naval cooks can prepare a good product with a minimum of attention and instruction, and finally whether the prepared product passes the ultimate and true test of taste acceptability with the ship's crew as the judges.

Should the food product pass these evaluations, specifications are cooperatively written by the interested services of the Defense Establishment and the product becomes an item for procurement.

Another facet of the work with foods is the preparation of provision loading lists, that is, a list of food supplies that must be loaded aboard ship. In this connection, it must be remembered that each ship has been designed for a primary operational function—combat, troop transport, or supply as examples—and, of necessity, the carrying and storage of food for feeding the crew is considered of secondary importance. This fact applies particularly to storage facilities with the consequence that the use of space and weight saving foods is important. A provision loading list should be prepared for each type of ship. However, the preparation of provision loading lists is further complicated by the fact that storage facilities vary from type to type and may even be different within the same type of craft. For example, the storage capacity of a destroyer markedly differs from that of an aircraft carrier and may even vary from that of other destroyers. An important nutritional consideration enters the problem in that the desires and the nutritional requirements of the crew vary with the climatic conditions — tropical, temperate, or frigid — and should be reflected by appropriate changes in the composition of the provision loading list.

The Commissary Research Division has also been working on a continuing project with industry in the preparation of the Navy Recipe Service. After being formulated with the help of industry and tested at the Commissary Research Division, the recipes are tested in the experimental kitchens of industry by members of the Navy Cookbook Task Committee of the National Security Industrial Association, and then are sent back to Commissary Research for check testing. At a meeting of the committee and Navy representatives, any recipe changes are ironed out and the recipes are approved for distribution to ships and shore stations.

In addition to the foods themselves, another area in which the Commissary Research Division concentrates its development activities is in the galley. Here, the effort is concentrated on both the equipment and the layout.

Upon being given the problem of a galley for a specific ship, studies of the situation are carried out by an industrial engineer. While industrial engineers have been used for many years by industry, particularly by manufacturing firms, the idea of employing industrial engineers to plan a concise and coordinated kitchen for food preparation is relatively new in mass feeding circles. The studies carried out by the industrial engineer are for the purpose of determining what types and how many pieces of a particular type of equipment are required. This initial study is prompted by the fact that many galleys in the past have lacked essential basic types of equipment and have been either over-sufficient or under-sufficient in what basic equipment was present. The industrial engineer also attempts to reduce the variety of utensil sizes, coordinate the size of cooking containers with the capacity of the basic heating and mixing devices, and provide planned, efficient stowage of equipment.

Once we know what equipment is needed to meet the situation, the problem becomes one of obtaining the proper equipment. At this point, it must be realized that equipment for mass feeding aboard ship is somewhat different from ordinary institutional feeding equipment in some important respects. When the Commissary Research Division has determined its equipment requirements to meet the problem, it turns to industry to supply the equipment. In many cases, no standard commercial item is available and a contract must be written for the manufacture of the article as desired.

Following the determination of needs and design of equipment, the industrial engineer lays out the galley equipment in a fashion designed to produce an efficient flow of work. If the problem involves a number of ships of the same type and galley area, a full size model of the galley may be built. In such a model or mockup, meals are prepared by Navy cooks in the proper amounts for the crew of the ship in question in order to test both the equipment and the feasibility of the layout.

Many pieces of equipment have been brought to the attention of the Commissary Research Division by manufacturers. If it is felt at Bayonne that the item has merit, the equipment will be tested there. Any equipment built according to the Commissary Research Division specification is also tested at Bayonne. If the item still appears to have merit but the tests indicate design modifications, the equipment is returned to the manufacturer for changes. An evaluation at sea then follows to determine if the item will perform as desired under actual operating conditions. Assuming a favorable evaluation at sea, the test results are sent to Washington for approval, following which the final specifications are written, and equipment becomes an item for procurement.

It is through the methods and approaches outlined that the Commissary Research Division endeavors to solve problems affecting Naval subsistence so that the men at sea can be kept at a high level of mental and physical proficiency under all types of adverse conditions.

Quick-Cooking Dehydrated Potatoes

W. O. HARRINGTON, R. L. OLSON, AND R. M. McCREADY
Western Regional Research Laboratory,[a] Albany, California

(Manuscript received April 16, 1951)

A method of preparing a quick-cooking dehydrated potato is described in which cooked pieces of potato are subjected to a controlled freezing and thawing operation before drying. Only standard processing equipment is required. The product can be prepared for serving by addition of hot water or other suitable liquid and stirring. Only a few moments are required for this operation.

The processing of potatoes to yield products that can be quickly prepared as mashed potatoes, as ingredients of soups, or in other ways, meets demands for (a) increased convenience of preparation, (b) improved storage stability, and (c) concentration by removal of water.

For many years technologists have sought means of improving dehydrated potato products and during the past fifteen years have developed a number of processes for dehydrated potatoes of instantaneous rehydration character *(7)*. A difficulty common to most of the established techniques is communition or extrusion of the product without undue rupture of tissue cells (which causes pastiness in reconstituted product) and drying of the particles without reclumping or uneven drying.

This report describes a method of preparing a quick-cooking, dehydrated potato product in non-granular form. Some of the principles employed in other processes are involved, but new applications are introduced that make possible the elimination of comminution or extrusion and allow use of conventional cabinet or tunnel dehydrators. This process involves controlled freezing and thawing of the cooked potatoes before dehydration.

Rivoche *(10, 11)*, Volpertas *(12, 13, 14)*, Greene, Marburger, and Rohrman *(3)*, Green, Robrman, Marburger, and Honstead *(4)*, Green, Conrad, and Rohrman *(5)*, Rendle *(8, 9)*, Willetts and Rendle *(15, 16)*, Barker *(1)*, and Barker and Burton *(2)* also found that freezing and thawing of cooked potato tissue can be used to advantage in preparation of dehydrated potato granules by the methods they developed. Greene et al. *(3)* stated that freezing at —4° C. (25° F.) is sufficient and that quick freezing adversely affects the ability of potato tissue to release water in the pressure-dewatering operation of their method. Furthermore, they reported that too rapid thawing renders cells more susceptible to rupture in subsequent operations.

Longrée *(6)* noted an adverse effect of slow freezing on the texture of frozen potato products. Microscopic observations showed a shrinking of vegetable cells when freezing rate was slow, and this effect was demonstrated to be related to a peculiar texture that was not desired. Subsequent reheating, however, reversed the condition and reswelled the cells.

[a] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

Studies at this laboratory on the characteristics of starch sponges [b] led to attempts to produce the sponge formation in situ in potato tissue. The product formed by dehydrating potatoes that have been cooked and subjected to slow freezing and thawing is sponge-like, like the heat-solubilized potato starch so treated. It is very porous and will soak up water rapidly. Rehydration is almost instantaneous, even in cold water, in contrast to the overnight soak or 30 minutes' simmer required for conventionally dehydrated potatoes.

Because of the appearance of the product and the relationship to the phenomena of starch-sponge formation, the term "sponge dehydrated potatoes" will be used throughout this report as a tentative designation for the quick-cooking dehydrated potato product herein described.

EXPERIMENTAL FINDINGS AND DISCUSSION

Technique of Preparation. Sponge-dehydrated potatoes have been produced by the following process: Potatoes of relatively high total solids contents were washed, mechanically peeled, trimmed, cut to half-dice size (3/16 x 3/16 x 3/8 inch), and cooked thoroughly on trays, loaded to about ¾-inch depth, in a steam blancher. After about 20 minutes of cooking, the product was fog-cooled and placed in a freezing room at —23° C. (—10° F.). Three to four hours later, when the product was frozen hard, the trays were placed in a room at 4.5° C. (40° F.) and allowed to thaw slowly over 12 to 16 hours. The trays of thawed product were then placed in a tunnel dehydrator at about 54.5° C. (130° F.) dry-bulb temperature and dried to approximately 8% moisture content in 3 to 5 hours.

Certain limitations of raw materials were encountered in the production of acceptable sponge-dehydrated potatoes. In general, the varieties characterized by high total solids content make the best mashed or sponge-dehydrated potatoes. Within varieties, the tubers of high total-solids content were best. An inferior product containing an appreciable number of pieces that do not rehydrate in a satisfactory manner will result if tubers of low specific gravity are used.

It is possible to sort potatoes by brine flotation, utilizing the correlation of high total solids with high specific gravity. By this means the best raw material for sponge dehydration can be selected for any given lot.

Control of cooking is also necessary in producing an acceptable product. Gelation of starch within the cells is essential to the formation of a sponge-like product. Cooking also softens the fibrous material, denatures protein, and alters flavor. Overcooking is apt to cause rupture of cell walls, with loss of quality. Under-

[b] Upon slowly freezing and thawing, heat-solubilized solutions of some starches form a stable sponge-like structure from which much of the water can be expressed by application of pressure. On drying, starch sponges will hold firmly together. They can be easily rehydrated up to about fifteen times their dry weight. (See Scharling, E. A., Annalen 49, 313 (1844); Woodruff and Hayden, *J. Agr. Research* 52, 233 (1936).

cooking does not produce a product that can be served without cooking after rehydration.

Cooking in water will cause some leaching of flavor and color, and vigorous boiling is apt to cause sloughing. Cooking with steam, on the other hand, gives a yellower, more flavorful, better-shaped, diced product, but some surface dehydration occurs and this seems to reduce porosity. This reduction in porousness appears to be due in part to surface dehydration before freezing (Figure 1).

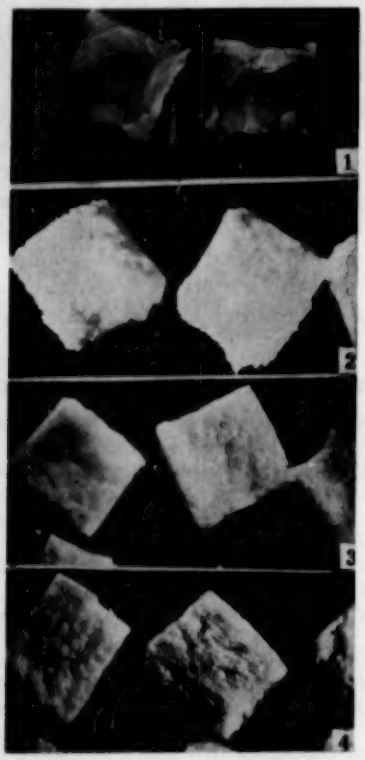


FIG. 1. Visual appearance of sponge-dehydrated potato. (1) Conventional dehydrated potato dice (3/16 x 3/8 x 3/8 inch); (2) cooked by boiling; (3) cooked by steam; (4) cooked by steam (surface scraped away to show porosity).

Cooking in steam and cooling with water, spray, or evaporative fog yields a product between the extremes produced by water and steam cooking. Twenty to thirty minutes of cooking have generally been found adequate, depending upon size of cut pieces, raw material, and conditions of cooking. Longrée *(6)* has reported discovery of a relationship between shrinkage or angulation of cooked potato cells on slow freezing and production of high-quality frozen potatoes. In the work reported here, slow freezing, or quick freezing followed by slow thawing, has caused a freezing-out of water from the solubilized starch, leaving a firm structure that will maintain its physical proportions throughout subsequent dehydration. The responsible action appears to be an egress of water from cell constituents into intercellular ice formations as the product passes slowly through a temperature range of approximately 0 to —2° C. (32 to 28° F.).

The slow passage through the critical temperature range can be accomplished by freezing at a slow rate, or freezing at a fast rate and thawing at a slow rate, or holding at the critical temperature for one to three hours. In the quick freezing, very small ice crystals will be formed and the product will have a much finer degree of porosity than in the slow freezing.

A manifestation of improperly controlled freezing and thawing is a non-uniform final product containing horny pieces with slow rehydration character. There appears to be an important relationship between character of raw material and control of freezing and thawing. These factors may make necessary extremely careful adjustment of processing conditions. In controlling the freezing or thawing rate, considerable adjustment is possible by alteration of conditions of heat transfer by such means as depth of tray loading, circulation of air, amount of exposed product surface, etc., as well as the temperature of refrigerant in freezing or heat source in thawing.

After thawing the water separated from the starch gel is not reabsorbed by the starch unless the temperature is raised to the point where gelation occurs (about 74° C. (165° F.). Because of this a shrinkage of the vegetable cells may be observed, as described by Longrée *(6)*, and microscopic examination of the product at this stage will enable an experienced person to predict whether or not an acceptable sponge-dehydrated product can be produced. This factor has been valuable as a control tool for experimental studies.

The thawed potato is dehydrated on trays in a tunnel or cabinet dehydrator. Product temperatures exceeding the temperature of gelation must be avoided. Because of the porous nature of sponge-dehydrated potatoes, the drying is more rapid than in conventional dehydration, and case hardening has not been observed in any of our studies.

Prior to dehydration, mechanical means of dewatering can be used. Direct or centrifugal pressure can be applied to reduce moisture content to about 45% and the press cake broken up and dried on trays. The density of the cake may slow the drying rate significantly but a less bulky product is produced.

Description of the Product. Potatoes have been dehydrated by the technique described above to obtain porous dice that are converted to mashed potatoes in a few minutes by addition of hot water or other liquid in about 5-to-1 weight ratio to the dry product. The product is seasoned and whipped or mixed thoroughly (Figure 2). While it is possible to rehydrate sponge-dehydrated potatoes with cold water, the use of water above 74° C. (165° F.) is required to cause swelling of cells and gelation of starch (Figure 3).

The sponge-dehydrated potato maintains virtually the same piece dimensions as cooked pieces subjected to drying. It is bulkier than most dehydrated potato products (Figure 4). An eight-ounce package requires about 150 cubic inches. Further, it is not possible to grind the product in the dry state to reduce its volume, since cell rupture would be unavoidable and hence a pasty product would result on rehydration because of release of starch. Even gentle crushing of dried dice causes the release of sufficient starch to produce an unpalatable product upon reconstitution. It is expected that this factor would be a limitation where shipping and storage space are critical, as in military rationing or other emergency use.

FIG. 2. Requirement for preparation of mashed potato from sponge-dehydrated potato dice. (1) Hot water 200 ml.; (2) sponge-dehydrated dice 50 g.; (3) milk 50 ml.; (4) seasoning and mixing.

Although porosity of individual pieces does not affect bulk density, it will vary somewhat and may be controlled to a considerable extent by alteration of processing variables. Even with careful control it is usually necessary to sort out a small number of hard pieces from the finished product.

The storage life of sponge-dehydrated potatoes parellels that of other dried-potato products. Since potatoes are bland in flavor, a very slight development of off-flavor is likely to be noticeable.

The product is relatively non-hygroscopic but will change slowly in moisture content with changes in relative humidity of storage.

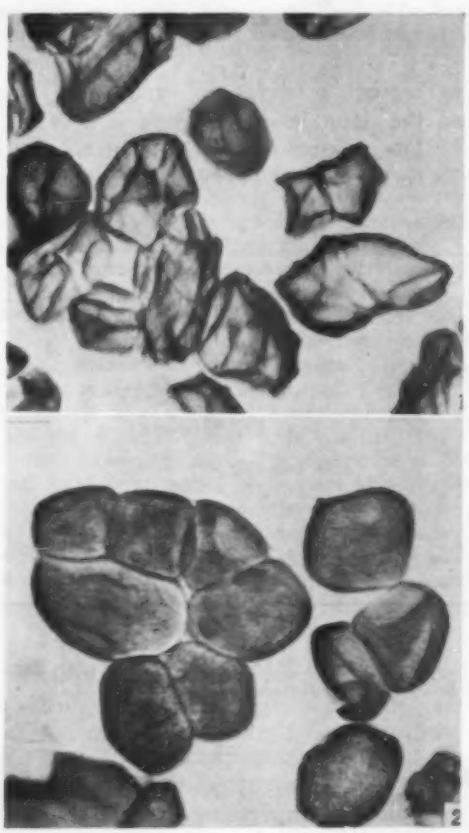


FIG. 3. Microscopic pictures of potato cells from sponge-dehydrated potato dice. (1) Potato cells in cold water; (2) potato cells in hot water.

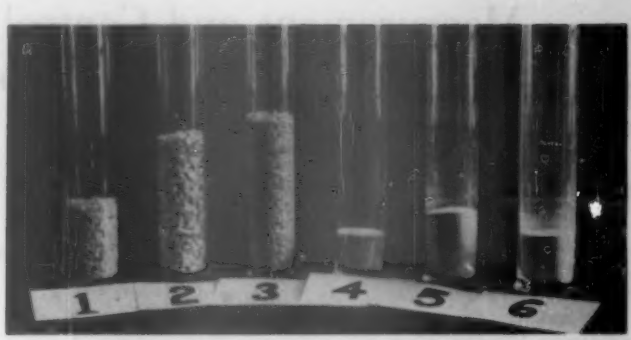


FIG. 4. Comparative volumes of equal weight for various dry potato products. (1) Conventional dehydrated dice; (2) sponge-dehydrated dice; (3) commercial dehydrated mashed potato, porous product; (4) commercial dehydrated mashed potato, granular product; (5) commercial dehydrated potato meal; (6) commercial dehydrated potato flour.

In a temperate climate, samples have been found acceptable after six months of storage in sealed cellophane bags with temperature ranging between 15.5 and 26.5° C. (60 and 80° F.). Under cold storage, or packaged in inert gas, a much longer shelf life is to be expected.

While this product is intended primarily for use as mashed potatoes and related dishes such as potato cakes, its use in soups, purees, casseroles, hash, chowder, etc., should not be overlooked. Eaten dry, the product has a crisp but tender texture. If treated with flavoring materials, it could well be used as a snack item.

LITERATURE CITED

1. BARKER, J. Improved process for producing starchy vegetable products in a powdered form. British Patent *542,125* (March 15, 1951).

2. BARKER, J., AND BURTON, W. G. Mashed potato powder. I. General characteristics and the "brush-sieve" method of preparation. *J. Soc. Chem. Ind. (London)*, **63**, 169 (1944).

3. GREENE, J. W., MARBURGER, G. C., AND ROHRMAN, F. A. Instant mashed potatoes from dehydrated granules. *Food Inds.*, **19**, 1622 (1947).

4. GREENE, J. W., ROHRMAN, F. A., MARBURGER, G. C., HONSTEAD, W. H., MESSENHEIMER, A. E., AND OLSON, B. E. Development of a potato granule process. *Chem. Eng. Prog.*, **44**, 547 (1948).

5. GREENE, J. W., CONRAD, R. M., AND ROHRMAN, F. A. Dehydrating process for starchy vegetables, fruits, and the like. U. S. Patent *2, 490,431* (Dec. 6, 1949).

6. LONGRÉE, KARLA. Quality problems in cooked, frozen potatoes. *Food Technol.*, **4**, 98 (1950).

7. OLSON, R. L., AND HARRINGTON, W. O. Dehydrated mashed potatoes. A review. Western Regional Research Laboratory, Albany, Calif., *U. S. Bur. Agr. Ind. Chem. Mimeographed Circ. Ser.* A1C **297** (Jan., 1951).

8. RENDLE, T. Improvements in and relating to the preparation of cooked starchy vegetables in powder form. British Patent *566,167* (Feb. 8, 1943).

9. RENDLE, T. Preparation of cooked starchy vegetables in powder form. U. S. Patent *2,381,838* (Aug. 7, 1945).

10. RIVOCHE, E. J. Improvements in and relating to the drying of vegetables. British Patent *601,151* (Sept. 16, 1939).

11. RIVOCHE, E. J. Drying of starchy foodstuffs. U. S. Patent *2,520,891* (Aug. 29, 1950).

12. VOLPERTAS, Z. Improvement in process and device for reducing vegetables containing starch to dry powder. British Patent *496,423* (Oct. 14, 1937).

13. VOLPERTAS, Z. Process for reducing vegetables containing starch to a dry powder. British Patent *525,043* (Feb. 13, 1939).

14. VOLPERTAS, Z. Art of dried starch bearing food. U. S. Patent *2,352,670* (July 4, 1944).

15. WILLETS, A. K., AND RENDLE, T. Improvements in and relating to the production of mashed potato powder. British Patent *589,876* (Nov. 7, 1945).

16. WILLETS, A. K., AND RENDLE, T. Production of mashed potato powder. U. S. Patent *2,439,119* (April 6, 1948).

Measurement and Control of Color in the Canning of Tomato Juice[a, b, c]

W. B. ROBINSON, J. R. RANSFORD, AND D. B. HAND
New York State Agricultural Experiment Station, Cornell University, Geneva, New York

(Manuscript received March 27, 1951)

When widely different chromaticities are not encountered, the a/b ratio of the Hunter Color-Difference Meter serves as an index of color grade of tomato juice. Prediction of the color of tomato juice from the color of raw tomatoes and the value of such prediction is discussed and the effects of processing conditions such as trimming, screening, preheating, and of pasteurization temperatures on tomato juice color are reported.

The use of PMA grades in the purchase of tomatoes for tomato products is a well established practice. The use of PMA standards for grades of tomato juice is becoming increasingly important, as purchases of canned tomato juice by government agencies are inspected and paid for on the basis of PMA grades.

In the PMA grades for tomato juice, color is a most important factor, since 30 points out of 100 are allocated to color. In addition, color is a "limiting factor," that is, a sample of juice cannot be graded as "Fancy" or "Grade A" if its color is rated below the minimum requirement for Fancy grade (26 points), however high the score for other factors. The other factors in grades, flavor, consistency, and absence of defects, can be largely regulated by careful control of operating and processing conditions.

In well-operated tomato juice canning plants, poor color is the cause for down-grading in the majority of cases. The importance of color in determining the grade of juice emphasizes, therefore, the need for accurate and objective methods for color measurement. The loss in financial return brought about by color down-grading is especially painful, since tomato juice with a Grade C color may cost as much to produce as with Grade A color.

The most widely used method for color measurement is the visual comparison method. The Maxwell spinning disc, calibrated according to the Munsell color system *(9)*, is used in industry as well as by federal graders. The Munsell Co., of Baltimore, Md., supplies papers for use on the disc and also color cards that demonstrate the color-grade limits of tomato products. Several solvent-extraction and chromatographic methods have also been suggested for the determination of the pigments responsible for tomato juice color *(2, 6, 15)*.

The most promising approach to objective color measurement is offered by various photoelectric methods which incorporate the tristimulus principle of color definition. Such methods include the use of the Beckman spectrophotometer *(7)* followed by integration of the curve according to the I.C.I. methods *(3)*. The Hunter Color-Difference Meter *(4)* has been used successfully for tomato products *(5, 12, 13, 14)*. This instrument makes use of filters devised to correspond to the I.C.I. tristimulus functions. The same filters are used in the Photovolt reflectance meter.

Of the methods tested in this laboratory *(10)*, the Color-Difference Meter was found to be the most rapid and accurate, and most easy to interpret. Reflectance measurements were made on juice in a sample container of optical quality Plexiglas, 2⅝ inches in diameter, filled to a depth of two inches. The standard reference tile, calibrated on the Beckman spectrophotometer, was within the range of tomato juice color. The Color-Difference Meter was used without the glass plate, using the L scale, the plastic block with 2¼ inches circular aperture, and small area illumination (about ⅝ x ⅜ inches). The ratio of the *a/b* readings in the tomato juice region of the color chart is directly proportional to I.C.I. dominant wavelength *(10)*. The chromaticity (analagous to saturation or Munsel chroma) is measured by $(a^2 + b^2)^{1/2}$, and the brightness is read directly as R_d or L. The dominant wavelength, and therefore *a/b*, is closely related to hue *(10)*. Since hue is the most important factor in determining color grade and since brightness is related to hue *(5, 9, 13)*, the *a/b* ratio can be used for grade estimation when chromaticities are similar. Seven hundred samples of tomato juice processed in the Geneva pilot plant from various grades and blends of raw material were graded by two federal inspectors.

The agreement between the inspectors' score for color and the Hunter instrument's *a/b* is shown in Figure 1. The calculation of grade from this set of data is given by the formula: Color score equals 7.18 + 12.05 *(a/b)*. A correlation coefficient of 0.964 was obtained for the data. The constants in this formula may differ with the method of processing and possibly also with varieties other than those tested in this study. The agreement between the instrument and the inspector

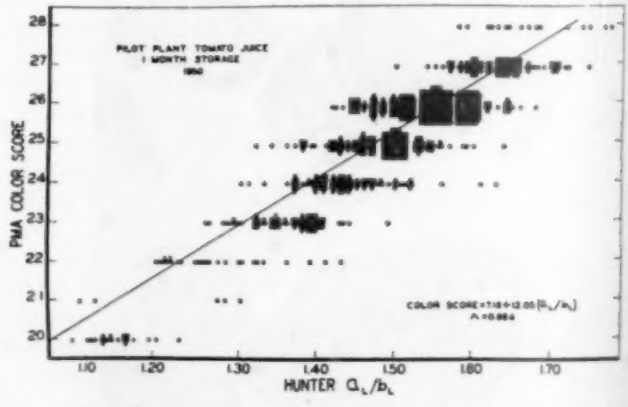


FIG. 1. Correlation of P.M.A. color grade with the *a/b* ratio of the Hunter Color-Difference Meter.

[a] Journal Paper No. 857, New York State Agricultural Experiment Station, Cornell University, Geneva, New York.

[b] This work was conducted as part of a cooperative project between N. Y. S. Agr. Exp. Sta., and the U. S. D. A. under Research and Marketing Act of 1946.

[c] Presented at the Eleventh Annual Meeting of the IFT, New York, N. Y., June 20, 1951.

was within one grade point in 85% of the samples tested.

When tomato juice samples of widely varying chromaticities are encountered the correlation between a/b, or dominant wavelength, and color score becomes lower. This is apparently caused by the interpretation of higher chromaticities as slightly yellower hues *(10)*, and should probably be considered when comparing juices processed under varying manufacturing conditions. Under such conditions a regression equation of the type: Color Score equals $K + Xa - Yb$ will be found more suitable *(5)*. However, for comparative evaluations of raw materials and processes in the same plant, the a/b ratio has been found sufficient, since variation in chromaticity has been found to be slight.

PREDICTION OF CANNED JUICE COLOR FROM RAW PRODUCT COLOR

The results of these studies were obtained in a small scale, continuous pilot plant with a capacity of one gallon per minute. All units were comparable to commercial type equipment *(8)*. The essentials of the manufacturing method consist of chopping, preheating to 77° C. (170° F.), finishing in a paddle-type finisher, pasteurizing at 121° C. (250° F.) for 0.7 minute, filling into No. 2 cans at 93° C. (200° F.), holding for three minutes, then rapid cooling to 37° C. (100° F.).

Under these constant processing conditions, the color of canned tomato juice may be predicted from the color of the raw tomatoes. Such a prediction is of assistance in quality control, especially when the juice is to be sold according to grade specifications. Thus an evaluation of the raw product would make possible the blending of highly colored loads of tomatoes with less ripe tomatoes, in proportions such that the maximum possible quantity of Grade A juice could be produced.

In order to study the possibilities of making such a prediction under practical conditions, raw tomato samples were taken from the pilot plant processing line. The tomatoes passed over the trimming table, fell through a chopper, and were then pumped to the preheater. In order to eliminate raw tomato sampling difficulties, the raw samples were taken from the small reservoir of chopped tomatoes immediately before the pump to the preheater. A 5 lb. composite sample was collected during the processing of each lot. The raw, chopped sample was passed through a laboratory tapered screw extractor (Chisholm-Ryder, Model F) fitted with an 0.040 inch screen. About 200 ml. of the extract was deaerated in a suction flask with the aid of a vacuum pump at about 10 mm. pressure. The deaeration procedure was used in all tests as an added precaution, although deaeration actually had little or no effect on the a/b ratio. The presence of a little air in the sample tends to change the a and b readings, and hence the chromaticity. The a/b ratio is not affected, however, unless large amounts of air are incorporated. High-speed finishers and extractors necessitate deaeration of the sample before color can be measured.

The relation between the colors of raw juice and canned juice is shown in Figure 2. The twenty-two points shown in the figure are average values for the various grades and blends of those grades that were repeated at weekly intervals during the six-week season. Table 1 shows the changes in the L, a, and b readings that occur during processing. An increase of 0.14 in the a/b ratio of the raw juice results in an increase of 0.10 in the finished product. (This is equivalent to about 1.2 grade points). Individual predicted color scores for finished juice were never more than the equivalent of one color grade point from the measured color of the finished juices.

The possibility of collecting a representative sample of tomatoes from a lot before processing was also investigated. Twenty tomatoes were collected at random from six baskets of each segregated grade of raw material on the grading platform. These tomatoes were extracted, deaerated, and the resulting raw juice was measured on the Color-Difference Meter. For comparison, samples from the same lots were collected from the

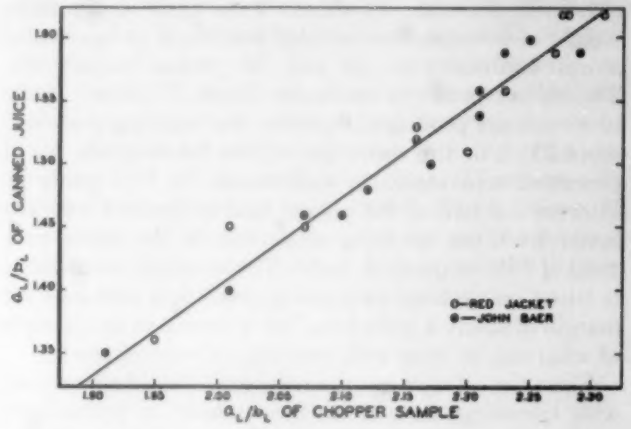


FIG. 2. The relation of the a/b ratio of raw tomato juice to the a/b ratio of canned juice.

TABLE 1

The effect of processing on tomato juice color

Lot number	Color before processing				Color after processing			
	L	a_L	b_L	a/b	L	a_L	b_L	a/b
Variety: Red Jacket								
1	25.0	21.8	9.6	2.27	28.2	21.6	13.6	1.59
2	25.7	20.3	9.8	2.07	29.0	20.5	14.1	1.45
3	25.2	21.7	9.5	2.28	28.2	21.9	13.5	1.62
4	25.1	21.9	9.5	2.31	28.0	21.7	13.4	1.62
5	25.5	21.1	9.6	2.20	28.6	20.9	13.8	1.51
6	25.2	21.6	9.7	2.23	28.3	21.2	13.6	1.56
7	25.4	21.2	9.8	2.16	28.6	21.2	13.9	1.53
8	26.5	19.5	10.0	1.95	30.0	19.9	14.6	1.36
9	25.6	20.8	9.6	2.16	28.6	21.0	13.8	1.52
10	25.5	22.2	9.8	2.27	28.3	22.0	13.6	1.62
11	28.9	17.3	11.3	1.53	32.1	17.7	16.1	1.10
12	26.8	20.7	10.3	2.01	30.1	21.2	14.6	1.45
Variety: John Baer								
1	25.8	22.7	9.9	2.29	28.8	22.1	13.9	1.59
2	26.9	20.7	10.4	2.01	29.8	20.7	14.8	1.40
3	25.8	21.9	9.9	2.21	29.1	21.6	14.0	1.54
4	25.8	22.3	9.9	2.25	28.5	22.1	13.8	1.60
5	26.1	21.7	10.0	2.17	29.4	21.5	14.3	1.50
6	25.8	21.9	9.9	2.21	29.0	22.0	14.1	1.56
7	26.4	21.6	10.3	2.10	29.4	21.0	14.4	1.46
8	27.5	20.1	10.5	1.91	30.7	20.3	15.1	1.35
9	26.7	21.1	10.2	2.07	30.0	21.0	14.4	1.46
10	26.0	22.7	10.2	2.23	28.8	22.4	14.1	1.59
11	28.9	18.4	11.6	1.58	32.7	17.8	16.3	1.09
12	26.4	21.6	10.2	2.12	29.9	21.6	14.6	1.48

chopper. The correlation of the color of the chopped tomato sample with the color of the canned juice for 21 lots of the Red Jacket variety was 0.97 while the correlation was 0.96 between the 20-tomato sample and canned juice. The corresponding coefficients for 18 lots of John Baer were 0.96 and 0.87; thus it was indicated that the 20-tomato sample was inferior to the chopped tomato sample in the case of the latter variety. The correlation obtained showed that a slightly improved technique for sampling whole tomatoes would make it possible to predict

the color of the finished juice. In commercial operations, when a lot of tomatoes may amount to 600 baskets, the difficulty of predicting color of finished juices from a random sample of raw tomatoes will be increased because of the difficulty of obtaining a representative sample. This operation also applies to the determination of official PMA grades on a load of tomatoes where the normal sampling procedure is to take four individual baskets for grading as representative of the load.

A separate prediction chart such as that shown in Figure 2, would be necessary for each individual plant since variations in pasteurizing temperatures, finishing equipment, etc., will change the constants of the regression line. An example of the use of such a prediction chart will illustrate its value. Two loads of the same weight of tomatoes are sampled and found to have color scores equivalent to 24 and 27 points respectively. Twenty-six points is minimum Grade A color. If the two loads are processed together, the resulting pack will score 25½, or the entire lot will be below grade A. If processed separately, the yield would be 50% grade A. However, if half of the poorer load is blended with the better load, the resulting score will be 26 points or a yield of 75% of grade A juice. The example, admittedly, is based on uniform processing conditions and cuts the margin of safety a trifle thin, but it serves as an example of what can be done with this type of control work.

Since the color of tomato juice may often be improved with trimming, as will be shown later, a preliminary measurement of the raw juice color may help to govern trimming severity. Tomatoes are trimmed primarily to control the mold count of tomato products. However, the color can be usually improved by trimming out color-deficient areas cause by immaturity or sunburn.

CONTROLLING PICKING STANDARDS

Color development in the tomato is influenced by the environmental temperature *(11)*. The internal color of the tomato fruit seems to lag farther behind the outer skin color when the field temperatures are lower. To study this effect the color of a random spot on the equator of each of 20 No. 1 tomatoes was measured twelve times during the season. Preliminary work showed that 20 tomatoes gave a reproducible average color measurement. The tomatoes were then extracted in the laboratory extractor (Seprosiv), and the color of the juice measured. The ratio of the juice color *(a/b)* to the average skin color *(a/b)* gives the relative redness of the internal tomato. The relation of this index to changes in season temperature is shown in Figure 3. These data also illustrate the well known fact that the outside color of a tomato is not the sole index for predicting the juice color. In practice, the grader attempts to predict the inside color by frequent cutting of the tomatoes and by taking softness into account. In colder weather, there is a tendency for the outside color to be relatively redder than the inside of the tomato. A sample extraction of the raw No. 1 tomatoes could serve as an indication of the relative redness of the internal tomato, and picking color standards might be raised or lowered accordingly.

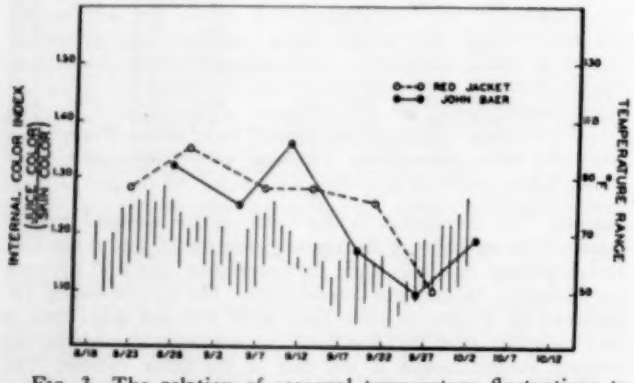


FIG. 3. The relation of seasonal temperature fluctuations to the internal color of ripe tomatoes.

EVALUATION OF PLANT PROCESSES

Tomatoes are trimmed before juicing in commercial practice, primarily to keep down the mold count and to improve flavor quality. In the Geneva pilot plant tomatoes were not trimmed purposely to improve color. However, careful trimming of tomatoes often improves the color of the juice, since mold spots, sunburn, and other similar defects are lacking in red color. Table 2 shows the improvement in color of tomatoes graded as No. 2 because of defects other than color deficiency. The raw juice color of untrimmed samples is compared to the color of corresponding samples after trimming. The average improvement in color is approximately equivalent to one point in the color grade.

TABLE 2

Effect of trimming on raw tomato juice color [4]

Date	Trimming loss,	Before trimming,	After trimming,	Difference
Variety: John Baer				
	%	*a/b*	*a/b*	
8/28	2.6	2.22	2.33	+0.11
9/6	6.4	2.20	2.37	+0.17
9/12	3.7	2.24	2.34	+0.10
9/19	12.2	2.09	2.21	+0.12
9/26	10.8	2.07	2.07	0.00
10/3	9.8	2.00	2.08	+0.08
Average				+0.10
Variety: Red Jacket				
8/31	8.0	2.14	2.33	+0.19
9/8	5.9	2.20	2.24	+0.04
9/14	7.9	2.29	2.34	+0.05
9/21	13.0	2.20	2.28	+0.08
9/28	8.3	2.00	2.00	0.00
Average				+0.07

[4] Juice made from tomatoes graded No. 2 because of defects other than color deficiency. Color expressed as *a/b*. Defects consisted chiefly of mold on surface and cracks, "cat-faces," etc.

Similarly, other factory practices and innovations may be evaluated from the color standpoint. Some plants use a vibrating screen in the tomato juice line immediately before the finisher. Theoretically, the screen is supposed to eliminate such defects as hard green spots and yellow shoulders. The waste from such a screen in the Geneva pilot plant was collected and extracted in the laboratory Seprosiv. A comparison of the color of the juice from such an extraction and that of the juice from the pilot plant finisher (Table 3)

shows that the use of the screen was actually deleterious to color during the greater part of the season. In the case of the juice made on August 28 and 30, the screen waste had poorer color in nine cases, and better color in eight. But as the season progressed, the juice color became poorer in relation to the screen waste. This effect is undoubtedly caused by the previously mentioned relationship between the internal-external color ratio and seasonal temperature. Since the waste from the screen is made up largely of skin and attached flesh, the screen will remove relatively more color when the internal parts of the tomato are not as well colored. On the other hand, in extremely hot seasons when the tomatoes are subject to higher incidence of sun-scald, it is possible that the screen might be of value. It should be kept in mind that the screen always reduces the yield of canned juice.

TABLE 3

Color comparison of juice with vibrating screen waste

Date	No. of lots	Variety	Av. waste color, *a/b*	Av. juice color, *a/b*	Difference
Aug. 23	6	Red Jacket	1.80	1.72	+0.08
Aug. 28	8	John Baer	1.97	1.98	−0.01
Aug. 30	10	Red Jacket	1.99	2.03	−0.04
Sept. 5	10	John Baer	1.92	1.87	+0.05
Sept. 8	11	Red Jacket	2.12	1.94	+0.18
Sept. 11	10	John Baer	2.07	1.95	+0.12
Sept. 14	11	Red Jacket	2.12	1.95	+0.17
Sept. 19	9	John Baer	2.05	1.87	+0.18
Sept. 21	11	Red Jacket	2.13	1.91	+0.22
Sept. 26	7	John Baer	1.98	1.75	+0.23
Sept. 28	8	Red Jacket	1.97	1.75	+0.22
Oct. 3	9	John Baer	2.04	1.79	+0.25

EFFECT OF HEAT ON TOMATO JUICE COLOR

Exposure to heat during processing adversely affects the color of tomato juice. For that reason more than the amount of heat required to eliminate harmful organisms present in the juice should be avoided. The effect of different temperatures of pasteurization on the color of tomato juice is shown in Table 4. Lot No. 1, when heated for 20 minutes at 93° C. (200° F.), had better color than a batch from the same lot when heated to 121° C. (250° F.) for only 0.7 minute. The latter conditions are recommended, nevertheless, in plants where spoilage from the thermoduric organisms may be encountered. With lot No. 2 a progressive deterioration of color occurred, with constant heating times, at temperatures increasing from 93 to 121° C. (200 to 250° F.). In the case of lot No. 3, the same observation was repeated with the highest temperature at 129.5° C. (265° F.).

TABLE 4

Effect of pasteurization temperature on the color of canned tomato juice

Lot no.	Pasteurization temperature, ° F.	Time in minutes	*a/b*
1	200	20	1.80
	250	0.7	1.74
2	200	0.7	1.88
	225	0.7	1.79
	250	0.7	1.74
3	200	0.7	1.63
	250	0.7	1.49
	265	0.7	1.46

In the above experiment, all lots were preheated to 77° C. (170° F.). It was thought that a study of the effect of various preheating temperatures followed by a constant pasteurization treatment would be of interest (Table 5). In series A lots, preheating temperatures of 60, 68, 77, 85, and 93° C. (140, 155, 170, 185, and 200° F.), were used. All lots were pasteurized at 250° F., for 0.7 minute. No significant differences were observed in the color of the finished product. With the lots of series B, preheating temperatures of 77, 93, and 101.5° C. 170, 200, and 215° F.), were used with the same result. This would appear to mean that the highest temperature reached during processing and the length of time at that temperature are the chief determinants of the finished juice color. However, this conclusion may only be valid under the conditions of processing used here, that is, under the preheating temperature and time used, with a high speed paddle-type finisher and high temperature pasteurization.

TABLE 5

Effect of preheating temperature on the color of canned tomato juice

Lot no.	Preheating temperature, ° F.	*a/b*
A 1	140	1.49
A 2	155	1.46
A 3	170	1.47
A 4	185	1.47
A 5	200	1.50
B 1	170	1.48
B 2	200	1.50
B 3	215	1.48

The effect of prolonged heat on the color of tomato juice was studied by immersing cans of tomato juice in a retort at 93° C. (200° F.), for periods of time up to eight hours. Duplicate cans were removed for each observation. Color was measured by the Color-Difference Meter and the samples were graded by an inspector (Figure 4). A decrease in all three color dimensions, *a/b*, brightness, and chromaticity, occurred under these conditions. The loss in color score was greater than was predicted by the change in *a/b*. This observation may illustrate the difficulty of visual grading. When one sample of a series is exceptionally good, the others may look comparatively worse than they really are.

Further studies on temperature effects on color and their relation to various methods of processing are planned for the 1951 season.

DILUTION OF TOMATO JUICE

In the course of studies on tomato juice color measurement, it was found that color of tomato juice depended to some degree on the penetration of light. In order to study this effect, samples of juice were diluted with water to 90, 80, 70, 60, and 50% juice

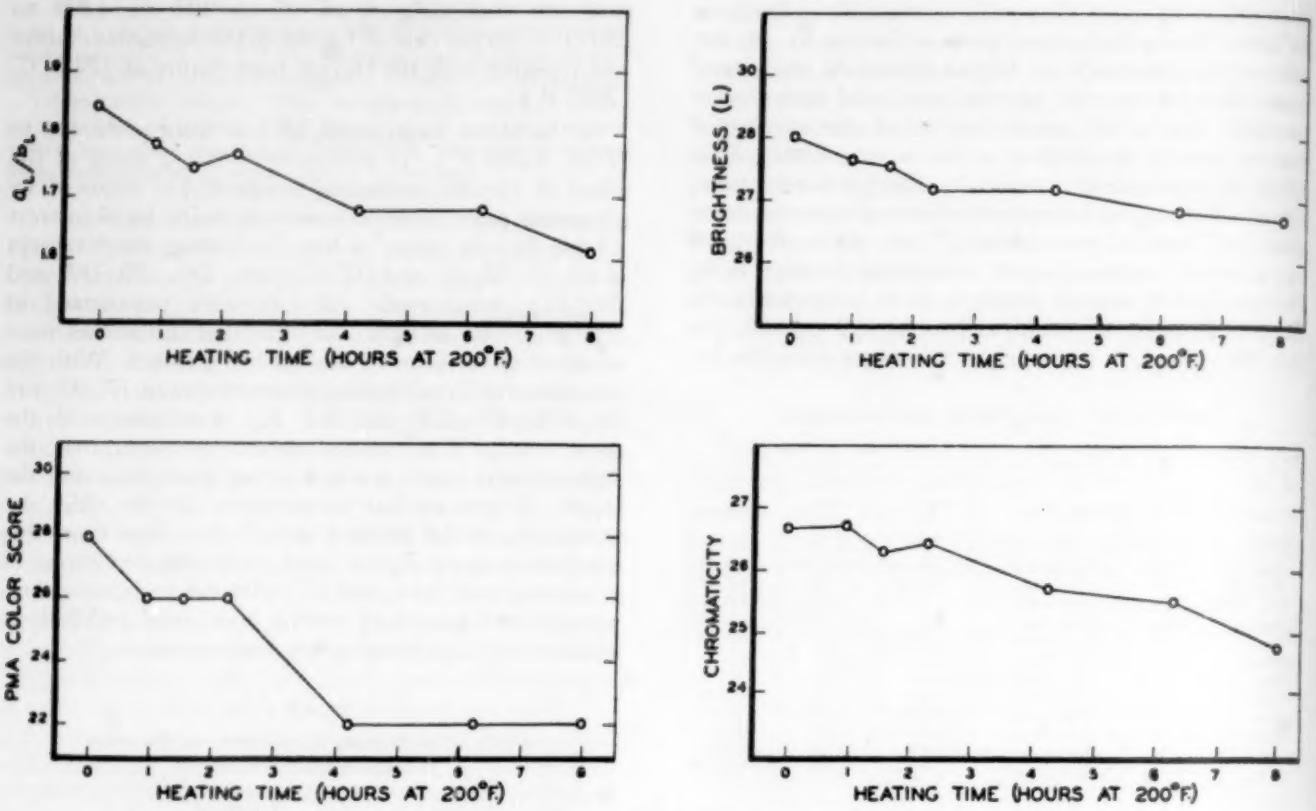


FIG. 4. The effect of holding at 200° F. on the color of tomato juice.

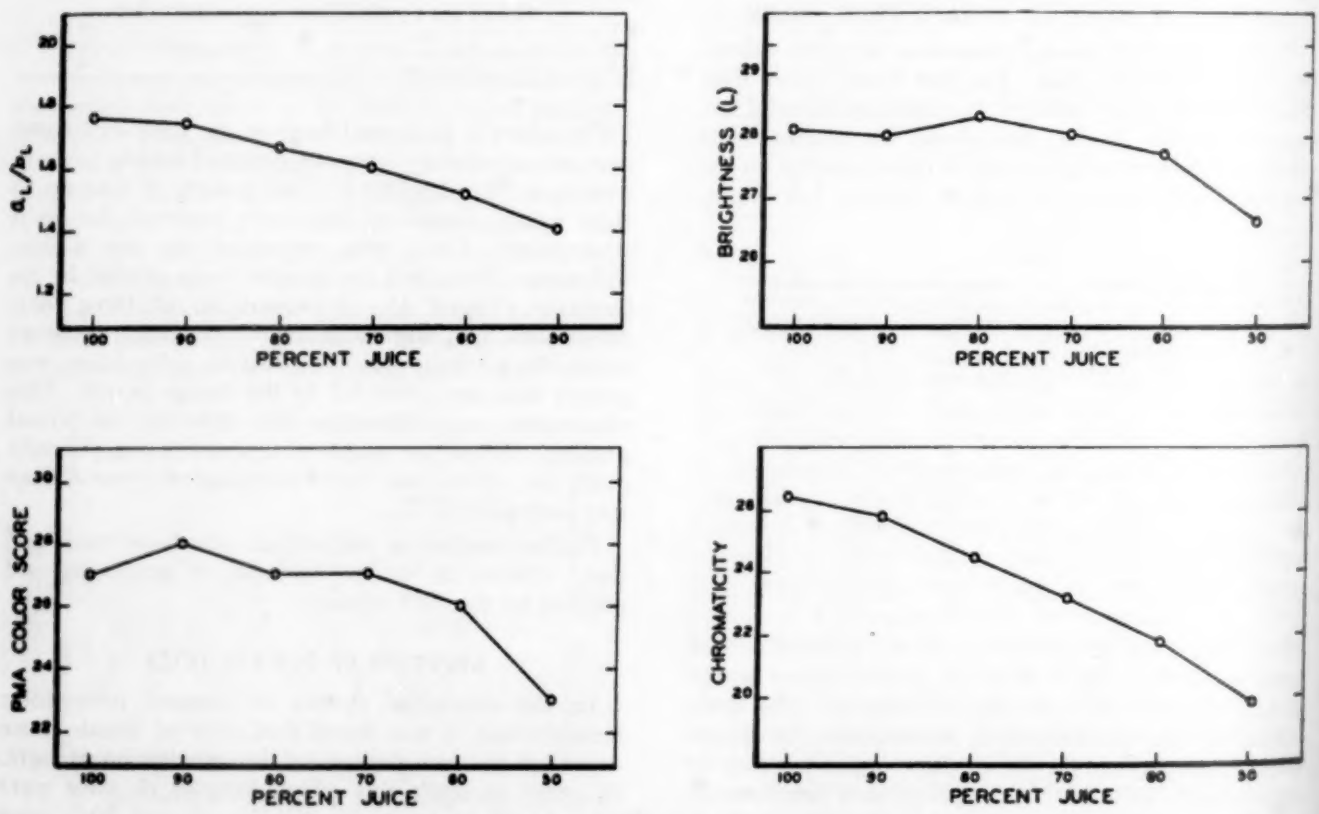


FIG. 5. The effect of dilution on the color of tomato juice.

(Figure 5). The three color dimensions decreased with dilution. The rate of decrease, particularly for the *a/b* ratio and brightness, accelerated with dilution. Brightness was not changed significantly until the 60% dilution was reached; the *a/b* index had changed significantly at 80%; and the chromaticity declined upon the addition of only 10% water. The decline in *a/b* index means that the juice becomes more orange in appearance. This observation was reported by Carrasco when he diluted samples of tomato juice before measuring them on a Lovibond Tintometer *(1)*.

SUMMARY

Tomato juice color can be determined with the use of an accurate, objective measurement method. When 700 samples were scored by federal inspectors and by the Hunter Color-Difference Meter, a correlation coefficient of 0.964 was obtained. Eighty-five percent of the scores agreed within one grade point.

Under uniform processing conditions, the color of canned tomato juice may be predicted from the color of the raw tomatoes. Such a prediction makes possible a control of the trimming operation or a blending of raw material to aid in standardizing the color of the tomato product, or to meet grade requirements.

Color development is influenced by environmental temperature, and during cool seasons internal color development does not keep pace with the external color. For this reason, tomatoes should be picked with a redder skin color during cooler weather.

Plant processes, such as trimming and screening, may be evaluated in relation to their effect on color. As pasteurization temperatures increase the juice color becomes slightly poorer. When juice was pasteurized at 121° C. (250° F.), for 0.7 minute, the preheating temperature used before pasteurization had no effect on color of the finished product.

LITERATURE CITED

1. Carrasco, O. Color of tomato products. *Ind. ital. conserve aliment*, **12**, 1 (1937). *C. A.* **31**, 3584 (1937).
2. Davis, W. B. Estimation of the color of tomato paste. *Anal. Chem.*, **21**, 1500 (1949).
3. Hardy, A. C. *Handbook of colorimetry*. The Technology Press, Cambridge, Mass. (1936).
4. Hunter, R. S. Photoelectric color-difference meter. *J. Optical Soc. Am.*, **38**, 661 (1948).
5. Kramer, A. This meter gives better color evaluations. *Food Ind.*, **22**, 1897 (1950).
6. Kramer, A., and Smith, H. R. Preliminary investigations on measurement of color in canned foods. *Food Research*, **11**, 14 (1946).
7. McCollum, J. P. Color and pigment studies with different grades of tomato juice. *Proc. Am. Soc. Hort. Sci.*, **44**, 398 (1944).
8. Moyer, J. C., Holgate, K. C., LaBelle, R. L., and Hand, D. B. The design and construction of a pilot plant tomato juice line. *Ind. Eng. Chem.*, (in press).
9. Nickerson, D. Color measurement and its application to the grading of agricultural products. U.S.D.A. *Tech. Bull.*, **154**. (1946).
10. Robinson, W. B., Clark, W. L., Wishnetsky, T., Kirchner, P., Ransford, J. R., and Hand, D. B. Instrumental methods for measurement of tomato juice color and evaluation of color grade. (Manuscript in preparation).
11. Vogel, A. C. Effect of environmental factors upon the color of the tomato and the watermelon. *Plant Physiol.*, **12**, 929 (1937).
12. Younkin, S. G. Application of the Hunter Color-Difference Meter to a tomato color measurement problem. *J. Optical Soc. Am.*, **40**, 265 (1950).
13. Younkin, S. G. Measurement of small color differences in tomato purees. *J. Optical Soc. Am.*, **40**, 596 (1950).
14. Younkin, S. G. Color measurement of tomato purees. *Food Technol.*, **4**, 350 (1950).
15. Zscheile, F. P., and Porter, J. W. Analytical methods for carotenes of *Lycopersicon* species and strains. *Anal. Chem.*, **19**, 47 (1947).

Experimental Treatment of Citrus Waste Water[a,b,d]

ROBERT R. McNARY,[c] RICHARD W. WOLFORD,[c] AND VINCENT D. PATTON[c]

Florida Citrus Experiment Station, Lake Alfred, Florida

(Manuscript received May 28, 1951)

Anaerobic methane fermentation appeared to be suitable as a treatment for citrus waste water, but factors arose that interfered with this type of fermentation. It was found that the essential oil from citrus fruit is toxic to these anaerobic bacteria. The essential oil can be eliminated from citrus liquors by allowing fermentation with naturally occurring yeasts to take place at the same time the liquor is aerated. After this treatment excellent results can be obtained by methane fermentation.

The treatment and disposition of waste water from citrus processing plants has been a difficult problem. The situation has changed little in Florida since it was outlined in 1947 *(8)*, with the exception that the volume of waste water has increased. The scattered geographical distribution of Florida citrus processing plants makes the disposal of wastes from each plant almost a unique problem. The many efforts to treat these wastes include lagooning, percolation through natural sand beds, drainage wells, septic tanks, and dispersion in salt water after preliminary fermentation with yeast. None of these methods has universal application and the use of drainage wells is no longer permitted.

At one canning plant partial elimination of nuisance has been achieved by segregating concentrated wastes and disposing of them in a remote area. The more dilute wastes pass through a ground level holding tank which must be skimmed continuously and the skimmings buried. The waste is then mixed with cooling water and passed through a shallow concrete flume, 1100 feet long, containing several low level dams to increase aeration

[a] Presented at the Eleventh Annual Meeting of the IFT, New York, N. Y., June 19, 1951.

[b] Cooperative research project of Florida Citrus Commission and Florida Citrus Experiment Station, Lake Alfred, Florida.

[c] Research Fellow, Florida Citrus Commission.

[d] Florida Agricultural Experiment Station, Journal Series No. 7.

and turbulence. The services of an operator are required at all times to obtain good results.

The treatment of domestic sewage sludge by methane fermentation has been successfully employed for 50 years and certain industrial wastes have been "digested" with methane bacteria for more than 20 years. The fact that a useful mixture of gases (methane and carbon dioxide) is produced in this treatment led the Florida Citrus Commission to begin laboratory investigations on the methane fermentation of citrus wastes in 1946, even though citrus wastes were different from materials found to be utilized by methane bacteria prior to that time. The initial work was conducted in cooperation with the U.S.D.A., but since 1947 it has been in cooperation with the University of Florida Citrus Experiment Station.

A methane fermentation differs considerably from more familiar fermentations in that the microorganisms are employed in a mixed flora. These methane-producing bacteria are found wherever organic matter is decomposing and can utilize a wide variety of plant and animal residues, which they break down into volatile organic acids as intermediates in the formation of methane and carbon dioxide. Methane fermentations are usually conducted continuously and will function from about 15.5° C. to 54.5° C. (60° F. to 130° F.), although the activity below 27° C. (80° F.) is quite low. A rather constant temperature is necessary nevertheless. These bacteria also require a certain amount of finely divided suspended solids (sludge) in the culture in order to carry out their metabolism. Various sugars, cellulose, starches, and proteins have been used experimentally as substrates.

EXPERIMENTAL PROCEDURE AND RESULTS

Initial Investigation of Methane Fermentation of Citrus Wastes. Initial results obtained by using a five gallon fermentation bottle maintained at 32° C. (90° F.) in a constant temperature cabinet, an inoculation of bacteria from a domestic sewage sludge tank of the Imhoff type, and spot samples of canning plant wastes or diluted orange juice as feed were disappointing. However, when the inoculation was made with sludge obtained from a starch waste treatment plant, gasification proceeded almost immediately.

Canned orange juice was diluted to approximately 1% dry solids and fed over a period of six hours to the fermenter at the daily rate of 0.13 pound of dry solids per cubic foot of fermenter volume (hereafter stated as lb./cu. ft.). The fermentation proceeded quite well under this regimen, except that the volatile acid concentration in the fermenting liquid fluctuated over a range of 300 p.p.m. to 1250 p.p.m. The amount of diluted juice added daily was increased in steps from 0.13 lb./cu. ft. to 0.16, 0.19, 0.23 and 0.26 lb./cu. ft. The daily rate at each step was held constant for one week before the next increase was made.

Upon attempting to increase the load on the fermenter to 0.29 lb./cu. ft. an error was made and 0.38 lb./cu. ft. was added. The volatile acid concentration then increased rapidly to 3000 p.p.m. and the fermentation went sour. The feed was stopped and the volatile acid concentration was thereby reduced; however, it rose again to high levels when the feed was resumed. Thereafter, the daily rate of feeding had to be restricted more and more to prevent excessive volatile acid formation. The reasons for this unique action of a methane fermentation have been the subject of investigation ever since it was first observed.

Other Factors Concerning Methane Fermentation. A culture of methane bacteria which had developed an excessive volatile acid concentration after being fed untreated orange juice for some time presented a microscopic appearance differing from a culture in good condition. A culture in good condition has a great predominance of short rod-shaped bacteria; whereas, in a culture which has developed excessive volatile acids a round coccus form is predominant. This raised the question of whether the predominance of one type or the other was brought about because the cultural conditions favored one type or whether the presence of the cocci brought about conditions unfavorable to the methane producing bacilli. The difficulty of growing a pure culture of methane bacteria prevented us from attacking this problem directly. Pasteurization of the feed for the methane fermentation and the addition of sodium metabisulfite and sulfur dioxide to the feed were tried in an attempt to limit the entrance of extraneous bacteria, but no particular improvement resulted.

The oxidation-reduction potential readings of a methane fermentation were taken several times a day for eight months. Periods of poor as well as excellent activity are included in these data. In general, the E_h was about —0.260 to —0.290 volt when the fermentation was in good condition and not very different when the fermentation was not in good condition. Attempts to alter the E_h by bubbling hydrogen through the culture or by electrolysis produced little change in the potential and had no effect on the fermentation. While methane bacteria are anaerobic, apparently they do not maintain in their cultures as low an E_h as some other anaerobes (—0.300 to —0.410 volt for *Clostridium botulinum* and *Clostridium tetani*). Furthermore, they do not seem to be sensitive to minor fluctuations in oxidation-reduction potential.

A fermentation operating satisfactorily at first, but which showed a progressive reduction in activity, might be suffering from a nutritional deficiency. The longer the fermentation progressed the more severe might be the deficiency. If due to an inadequate supply of certain minerals, the difficulty could be easily corrected by mineral supplements. In particular, those mineral salts which tend to raise the pH and increase the buffering capacity might be advantageous, since a decreasing pH is one of the characteristics of a "sick" fermentation. To test this premise the following materials were added in varying amounts to the substrate: hydrated lime, calcium carbonate, calcium acetate, sodium sulfide, ferric chloride, sodium carbonate, sodium hydroxide, and ammonium hydroxide. Several times a temporary improvement for two or three days took place when the minerals were added. This probably indicates that the minerals as they occur in the waste water are not ideally proportioned. Such an addition, then, probably improved their interrelationship but did not alter the basic difficulty, since no permanent improvement was noted.

It was suggested by Dr. A. M. Buswell that the fermentation substrate might not contain sufficient finely divided suspended solids to meet the requirements of the methane bacteria. To test this suggestion rigorously, whole citrus pulp was subjected to methane fermentation. Ground fresh oranges, heavily inoculated with active sludge, were fermented in a specially designed

vessel similar in construction to a screenings digester *(3)* which allowed adequate mixing and dispersion of the fermenting mixture. The fermentation started off very well and the feed was increased until 0.19 pound of dry solids per cubic foot of fermenter volume was being added daily. This level could not be maintained, since the volatile acid concentration increased sharply. This procedure was repeated two more times with very similar results. The amount of suspended material present was certainly adequate in these trials for it was never less than 30% by volume.

After these extensive investigations concerning such factors as volatile acid production, oxidation-reduction potential, mineral and sludge requirements as well as proper temperature, agitation, and rate of feeding, it was obvious that citrus liquids and pulp were not suitable substrates for methane bacteria without some kind of preliminary treatment.

Methane Fermentation of Citrus Still Slops. Since other workers *(4)* had successfully used methane bacteria to "digest" still slops resulting from the production of alcohol from grain, it seemed likely that still slops derived from the production of alcohol from citrus press liquor or citrus molasses might act similarly. Citrus still slops were found to ferment with methane bacteria readily, the main difficulty encountered being a strong tendency to foam. However, a certain batch of slops caused the volatile acids to rise just as orange juice had in the past. Analysis of the slops showed 0.65% sugar remaining from an incomplete alcoholic fermentation. The methane fermentation recovered when a batch of slops containing a negligible amount of sugar was used. These results focused some suspicion on sugar as a toxic factor in the methane fermentation of citrus materials. More important, however, these results led to a preliminary treatment for orange juice which permitted unhindered methane fermentation.

Since complete removal of sugar might be necessary, a further study was made to determine the most efficient manner to accomplish this by yeast fermentation. Baker's yeast, *Torula utilis*, and naturally occurring wild yeasts were tried. Best results were obtained with the indigenous yeasts when a stream of air was blown through the liquid and in most of the investigations described below the following procedure was used: A suitable quantity of orange juice adjusted to 8% soluble solids (by Brix hydrometer) was aerated at the rate of 0.05 cu. ft./min./gal. After 24 hours half of this liquid was withdrawn and replaced with an equal quantity of fresh juice also containing 8% solids. Each day this operation was repeated. After three days a very active yeast culture developed and the sugar concentration in the withdrawn liquor was reduced to 0.01-0.03% as measured by chemical analysis. The dry solids in this liquor averaged 1.80%.

When this low-sugar, aerated juice was fed to a laboratory-size methane fermenter a flourishing activity followed. The gas was rich in methane (about 75%) and the volume was more than could be accounted for on the basis of the dry solids in the feed. Analysis showed that the yeast had produced appreciable amounts of alcohol which in turn was converted into methane and carbon dioxide by the bacteria. This two-stage treatment of orange juice diluted to 8% solids for uniformity was maintained for a total period of seven weeks. Part of this period was used to attain the high activity indicated. All of the data are not presented because mechanical difficulties such as gas leaks and electric current interruptions produced fluctuations in the data from outside influences. The following summary is typical of 13 days' good operation and the figures are averages of daily results over this period:

Methane fermenter loading, lb. dry solids per cu. ft. of liquid capacity per day, alcohol not included	0.22
Alcohol (% by weight) produced in aerobic stage	1.78%
Overall reduction in dry solids (both stages)	91.4%
Reduction in dry solids in aerobic stage, percent of overall reduction	49.0%
Reduction in dry solids in anaerobic stage, percent of overall reduction	51.0%
Reduction in dry solids in anaerobic stage, based on solids entering this stage	83.1%
Gas production in anaerobic stage, cu. ft. per lb. dry solids plus alcohol entering this stage	16.15
Daily gas production in anaerobic stage, cu. ft. gas per cu. ft. of fermenter volume	7.35

These results are very satisfactory. The daily volume of gas produced was seven times the liquid volume of the methane fermenter, as compared with a sludge digester in a sewage treatment plant in which gas production is only one-half its liquid volume per day, or one-fourteenth the amount obtained in this study.

Inhibitory Action of Citrus Peel Oil on Methane Fermentation. Orange juice previously fermented while being aerated was adopted as a suitable basic feed for a series of experiments designed to reveal substances suspected of inhibiting methane fermentation. The daily basic feed was composed of three doses sufficient to provide 0.09 lb./cu. ft. and a vigorous methane fermentation ensued. The major constituents of citrus juices, dextrose, sucrose, citric acid, pectin, and naringin (from grapefruit juice) were then added as supplements dissolved in the basic feed. None of these materials interfered with the methane fermentation; rather, they were all utilized in producing gas. The maximum amounts added were 0.05 lb./cu. ft. for the sugars and 0.03 lb./cu. ft. for the other supplements.

These results dispelled any suspicions that the presence of appreciable amounts of sugar was antagonistic to a good methane fermentation. However, as a confirmation, it was decided to try concentrated orange juice as a supplement. The concentrate used was produced by evaporating fresh juice at a low temperature, 21° C. (70° F.), under vacuum until it reached a concentration of 55° Brix. Surprisingly, the volatile acid concentration started to rise almost as soon as this concentrate was fed to the methane culture as a supplement in the aerated, fermented basic feed. This seemed to nullify our previous findings that additions of the major juice constituents did not retard fermentation. However, these supplements did not include citrus peel oil and an analysis showed that a small, but definite, quantity of peel oil remained in the concentrate. Suspicion

was therefore focused on the essential oil as the inhibitor. Definite traces of peel oil have also been found in citrus molasses made by the evaporation of press liquor. Apparently evaporation alone does not completely eliminate all of the essential oil in citrus liquids.

A new fermentation was started using the same basic feed; that is, orange juice diluted to 8° Brix and fermented with naturally occurring yeast while being aerated. Analysis of this basic feed indicated no detectable essential oil present. When the methane fermentation became sufficiently active to be fed the basic ration at the rate of 0.09 lb./cu. ft., d-limonene, the principal constituent of orange oil, was added as a supplement at the rate of 1 ml. per day. This was equivalent to 0.055% by volume of the daily feed. After eight days no signs of inhibition were apparent. However, when the amount of d-limonene was then raised to 6 ml. per day, inhibitory symptoms appeared in three days. In four more days the volatile acid concentration exceeded 1900 p.p.m., even though the addition of d-limonene was stopped. Analysis of the methane culture gave 0.069% oil which accounted for approximately half the di-limonene added as supplement. The outstanding conclusion derived from this experiment was that citrus peel oil contains one or more substances inhibitory to methane bacteria.

The employment of a basic feed plus supplements had been useful in locating the inhibitor in citrus juices. The basic feed had itself undergone a yeast fermentation with aeration. In order to demonstrate the effect of aeration without concurrent yeast fermentation, two five-gallon carboys were filled with pasteurized orange juice; one bottle was aerated until barely detectable quantities of essential oil remained and the other was held at the same temperature but unaerated. Two methane fermentations were fed from these two bottles at the level of 0.20 lb./cu. ft. No appreciable difference between the two fermentations was noted for the first 16 days. The daily level of feed was then stepped up to 0.25 lb./cu. ft. The fermentation being fed the aerated juice continued to accept the increased feed without difficulty and produced a correspondingly increased amount of gas. However, the fermentation being fed the unaerated juice developed a steadily increasing volatile acid concentration. In ten days the acids had reached 3110 p.p.m., at which point this fermentation was concluded; this fermenter liquor contained 0.010% essential oil. The fermentation being fed aerated juice was continued for an additional seven days with no deterioration in its activity. However, when the feed of this aerated juice was increased to 0.30 lb./cu. ft. it, too, showed symptoms of inhibition. Analysis of this fermenter liquor gave 0.005 percent essential oil. Apparently then, simple aeration did not completely remove the essential oil in this instance and the quantity of oil present determined the time of appearance of the inhibitory symptoms.

DISCUSSION

In developing a practical means of treating the waste waters from citrus processing plants the special characteristics of these wastes must be given primary consideration. In addition to containing essential oil, citrus wastes generally have a pH between 3.5 and 4.0. Furthermore, about two-thirds of the solids consist of the sugars dextrose, levulose, and sucrose. These properties favor the growth of yeasts until the sugars are consumed and during the yeast growth the liquor becomes more acid due to the production of acetic and other organic acids. The resulting liquor is then difficult to treat by customary procedures because of its low pH and concentration of organic substances.

In the beginning it was hoped that citrus wastes could be "digested" with methane bacteria, such as has been subsequently done with pear wastes *(7)* without preliminary treatment. When this was found impossible, much time and effort was expended to find an explanation. After supplying all the known conditions for a healthy fermentation, such as proper temperature, adequate sludge, E_h, agitation, mineral requirements, and feeding schedule without producing satisfactory results, the most reasonable explanation was that an inhibitory substance was involved. The essential oil, when present in appreciable amounts, might well be toxic to methane bacteria, since it contains some citral which is known to be bacteriostatic *(1)*. However, when citrus molasses was used as a substrate (after dilution with water) typical inhibitory symptoms appeared. This material is made by the evaporation of pressed citrus peel liquor. It is concentrated from 10 to 12% solids to 72% solids. One would expect that all of the essential oil would be removed by this operation. Indeed, an analysis by the officially recognized Clevenger method *(5)* indicated no oil present. However, Curl *(6)* has pointed out that the Clevenger method is inaccurate for low concentrations of oil. It was only after the aeration step had been included that the more sensitive Burdick and Allen procedure *(2)* for determining essential oil was used, and it was only with this procedure that essential oil was found in citrus molasses.

It has been mentioned that still slops remaining from the production of alcohol from citrus molasses and press liquor were fermented satisfactorily until a certain batch of slops was encountered. The use of this batch of slops as feed caused typical inhibitory symptoms to develop in the methane fermentation. The slops contained 0.65% sugar, indicating that the alcoholic fermentation had not completed itself. Unfortunately no essential oil determination was performed on this batch of slops. The entire history of the slops is not known to us. It is known that the yield of alcohol was low. It is our belief that there was sufficient essential oil present to prevent the completion of the alcoholic fermentation and enough remained in the slops to inhibit the methane fermentation.

It has now been amply demonstrated that citrus juices which are fermented with yeast and aerated until the peel oil is eliminated are satisfactory substrates for methane bacteria. There is some indication that aeration and heating may alter the toxicity of the essential oil to some extent by oxidation, as well as provide for the elimination of it by volatilization. The rapidity with which the very small amount of peel oil in the 55° Brix orange concentrate showed its inhibitory effect was out of proportion to the amount present (0.0125%). This

concentrate had not been heated above 21° C. (70° F.) and had very slight contact with air during extraction of the juice. Juices with equally small amounts of oil present usually require a much longer time for the inhibition to appear. Perhaps concentration of toxic constituents in the residual oil occurs during evaporation.

The question has been raised whether the necessity for pretreatment with air does not eliminate methane fermentation as a practical treatment for citrus wastes. It is the authors' opinion that methane fermentation after yeast fermentation with aeration has a definite place in the treatment of citrus waste waters. It will effectively utilize both the organic acids and the particulate matter (yeast cells and pulp) in making gas. Alternative secondary treatments might be the use of an activated sludge process, or of trickling filters such as used in sewage treatment. Trickling filters will not handle a liquid containing appreciable quantities of settleable solids because the latter will clog the filter. These solids might first be removed and treated separately by methane fermentation. The activated sludge process will handle suspended solids satisfactorily but thus far we have not been able to develop an activated sludge culture on citrus wastes. However, the amount of effort in this direction has not been sufficient to abandon this possibility.

SUMMARY

Citrus liquids and waste waters can be successfully fermented with methane bacteria only after all the citrus peel oil is eliminated. Yeast fermentation with aeration is a suitable means of removing the oil. The treatment of citrus wastes based on this information is being developed.

LITERATURE CITED

1. Bose, S. M., Bhima Rao, C. N., and Subrahmanyan, V. Some factors affecting the bactericidal property of lemongrass oil emulsions. *J. Sci. Ind. Research*, (India) **9**, (1) 12 (1950).
2. Burdick, E. M., and Allen, J. S. Rapid estimation of citrus peel oil. *Anal. Chem.*, **20**, 539 (1948).
3. Buswell, A. M., and Boruff, C. S. U. S. Patent *1,880,772* (Oct. 4, 1932).
4. Buswell, A. M., and Hatfield, W. D. Anaerobic fermentations. Illinois State Water Survey *Bull.*, **32** (1941).
5. Clevenger, J. F. Determination of citrus oils. *J. Assoc. Offic. Agr. Chemists*, **17**, 371 (1934).
6. Curl, A. L. Comparison of several types of apparatus devised for the determination of volatile oil in citrus juices. *J. Assoc. Offic. Agr. Chemists*, **30**, 567 (1947).
7. Duggan, Kenneth E. Pilot plant production of methane by anaerobic fermentation of pear waste. M.S. Thesis, Oregon State College, Corvallis, Oregon (1950).
8. McNary, Robert R. Industrial wastes, citrus canning industry. *Ind. Eng. Chem.*, **39**, 625 (1947).

Role of Glucose in the Storage Deterioration of Whole Egg Powder. III. Effect of Glucose Removal Before Drying on Organoleptic, Baking, and Chemical Changes

LEO KLINE, HELEN L. HANSON, T. T. SONODA, JOAN E. GEGG, R. E. FEENEY, and HANS LINEWEAVER

Western Regional Research Laboratory,[a] Albany, California

(Manuscript received June 2, 1951)

Removal of glucose from dried whole egg markedly stabilizes baking quality and flavor of the product. Browning is practically eliminated and the extent of various chemical changes is greatly reduced. In the normal glucose-containing whole-egg powders, loss in baking quality is associated chiefly with the occurrence of the glucose-protein reactions, while the glucose-cephalin reaction plays a major role in off-flavor development.

The joint efforts of a number of collaborating laboratories in England, Canada, and the United States, during World War II, resulted in a whole-egg powder of stability definitely improved over that available at the start of the war. Modifications in processing and packaging methods contributing to this improvement included more sanitary handling of the liquid melange, preheating or pasteurization before drying, production of low-moisture (2%) powders, rapid cooling of the powders after drying or redrying, and packaging of the powders in inert gases in hermetically sealed containers. However, these measures did not adequately protect this improved product from rapid flavor deterioration when it was subjected to moderate to elevated storage temperatures 30 to 38° C. (85 to 100° F.). The present report represents results of an attempt to elucidate the nature of the reactions responsible for this "high-temperature" type of flavor deterioration.

Reports of previous investigations suggested that certain egg components might be directly involved in these reactions. In 1944 Hawthorne and Brooks in England

[a] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

reported a striking stabilizing effect of glucose removal on the chemical and functional changes associated with the protein constituents *(8)*. Unfortunately their yeast-fermentation method for removal of glucose prior to drying introduced excessive foreign flavors and hence did not permit evaluation of the effect of glucose removal on flavor stability. Two years later, investigators at the Western Regional Research Laboratory reported evidence for an association of off-flavor development with changes in the phospholipid components *(1, 5)*. These changes were thought to result from a reaction between the phospholipid, cephalin, and aldehydic substances arising from oxidative decomposition of the lipids. The evidence for this contention was the demonstration that increases in browning, fluorescence, and ultraviolet absorption, which occurred during the high-temperature storage of whole-egg powder in air, could be approximately reproduced in a model system reaction between freshly prepared cephalin and acetaldehyde *(4)*. Furthermore, increase in lipid fluorescence, applied as a measure of this reaction, was reported to be correlated with decrease in palatability *(1)*.

These reports suggested that glucose-induced storage deterioration, though important from a functional standpoint, bore little relation to off-flavor development. However, a more recent report by some of the present authors *(10)* has shown that glucose is the principal reactive aldehyde involved in the cephalin deterioration and that oxidation is therefore not necessary for this deterioration to proceed.

The present paper describes the gross effects of glucose removal before drying on organoleptic and functional stabilities and also discusses the correlation of these effects with chemical manifestations of glucose-induced changes, particularly the glucose-protein and glucose-cephalin interactions.

METHODS

The yeast fermentation process employed for glucose removal, and the preparation of the whole-egg powders used in the storage experiments, have been previously described *(9)*. For each experiment all the powders were derived from the same batch of parent melange. Powders were stored either in sealed vacuum-type desiccators or in hermetically sealed cans. In the latter case, No. 2 cans containing 8 ounces of powder each were generally used.

Gas analyses for CO_2 and O_2 in the cans following storage were performed with the aid of a special can-puncturing device attached to an Orsat apparatus *(15)*. The CO_2 and O_2 were absorbed in potassium hydroxide and alkaline pyrogallol, respectively. The measuring burette used in the Orsat apparatus was calibrated in divisions of 0.2 ml. and permitted easy interpolation to 0.1 ml. on a total gas volume of 100 ml.

The organoleptic evaluation results were based upon the mean scores assigned by a trained taste panel of 6 to 7 judges to "scrambled eggs" prepared from the reconstituted powders. The reconstituted liquids were heated, with stirring, on a steam bath until firmly coagulated and were tasted without added seasoning. Judges were instructed to grade only for degree of storage off-flavor and to ignore color and texture differences, if possible. Color differences were found to be minimized by judging under sodium vapor lamps. Scores were assigned on the following basis: 10, no off-flavor; 8, slight but definite off-flavor; 6, pronounced off-flavor; 4, very pronounced off-flavor; 2, intense off-flavor; and 0, extreme off-flavor. Most of the experimental samples were judged according to the statistical plan known as the balanced incomplete block design. The applicability of this plan to organoleptic evaluation of egg powders has been reported by Hanson, Kline, and Lineweaver *(6)*. Under this plan as many as 10 samples were compared in one experiment. These samples, which included controls, were coded so that at any particular judging period the judges received, for example, only 4 of the 10 samples and were not aware which ones they were receiving. Each sample was replicated several times, but each time in a different block of 4. The final mean scores were treated by analysis of variance.

Baking quality performance was judged by the preparation of sponge cakes. In the method used, the batters were prepared by whipping at 40° C. (104° F.). The results reported should not be construed as indicative of the potential performance of egg powders in sponge goods not containing an added leavening agent, since whole-egg powders produced commercially by spray-drying are known to retain practically none of the room-temperature whipping properties of fresh egg. The cakes were made with the following formula: reconstituted egg, 70.4 g.; cream of tartar, 0.33 g.; sugar, 49.4 g.; flour, 28.3 g.; salt, 0.33 g.; and vanilla, 0.5 g. Dried egg was reconstituted by adding 3 parts of water and shaking vigorously for 15 minutes on a mechanical shaker. The egg, sugar, salt, and cream of tartar were placed in the bowl of a Hobart Electric Mixer, Model K4-B. The beater and bowl were placed in a water bath at 50° C. (122° F.), and the egg mixture was mixed on speed 1 until the sugar dissolved and the mixture reached a temperature of 40° C. (104° F.). The mixture was then whipped 400 seconds at speed 10. Final temperature was 40° C. ± 1°. The vanilla was then added followed by the sifted flour added in thirds and folded in by hand with a flat wire whip. The specific gravities of the foam and batter were recorded. Portions of 115 g. of batter were weighed into small rectangular cake pans (5½ x 3½ x 2¾ inches) and cakes were baked for 20 minutes at 175° C. (347° F.) in a reel-type cereal chemist's test oven. Cakes were inverted and cooled at least one hour before the volume was measured by seed displacement. Duplicate cakes were prepared in every case and mean measurements are reported.

The methods used for separation of the lipid fractions from the whole-egg powders and the spectrophotometric and fluorometric measurements on these fractions have been previously described *(10)*. For the determination of salt-water-soluble fluorescence, a modification of the procedure of Pearce, Thistle, and Reid was used *(12)*. The modification consisted chiefly in use of the ether-extraction residues from the Soxhlet-extracted whole-egg powders in place of the fat-free residues prepared by room-temperature chloroform extraction. Since the temperature of solvent extraction does have an effect on the salt-water-soluble fluorescence values obtained *(12)*, this procedure resulted in higher values for the controls. However, this was not considered important, because the samples were being evaluated on a comparative rather than on an absolute basis. The salt-water-soluble fluorescence results are reported according to the function $\Phi = (100\ I_s/I_{qu})/C$ where $I_s/_{qu}$ is the ratio of fluorescence intensity of the sample to that of the standard quinine solution and C represents the grams of fat-free residue extracted per liter of solution *(2)*. Glucose was determined according to the method of Somogyi *(14)*. The pH of the powders was measured with a pH meter on 1 g. of egg powder reconstituted rapidly in 3 ml. of CO_2-free water. The "solubility" of the egg powders was determined according to method No. 1 of Hawthorne *(7)* and the figures reported represent the percentage of the total nitrogen in the egg powder which was filterable through Whatman No. 12 folded filter papers after reconstitution in 10% KCl. The values are reported in the tables as "Protein Solubility."

RESULTS

Studies were made on the organoleptic, functional, and chemical stabilities of whole-egg powders under varying conditions of moisture content and gas pack. For any chosen set of conditions, comparisons were made of the stabilities of a glucose-free and an untreated (glucose-containing) powder prepared in each experiment from the same parent whole-egg pulp.

TABLE 1

Effect of glucose removal on off-flavor development during storage at 37.5° C. (100° F.)

Experiment no.	Sample		Palatability scores [b]						Difference required for significance at 5% level
			Storage time at 100° F.						
	Moisture content, %	Stored in	1 mo.		2 mo.				
			U [c]	F [c]	U	F	U	F	
1. Lyophilized powders. Fermentation with *T. monosa*	(Control) [d]	.....	9.7	9.7	9.7	9.2	....	....	Preliminary experiment. Not judged statistically.
	2	N_2	6.3	9.4	6.0	9.3	....	....	
	2	air	5.0	7.4	4.8	7.0	....	....	
			1 mo.		3 mo.		6 mo.		
6. Lyophilized powders. Fermentation with *T. monosa*	(Control)	.....	9.8	9.6	9.6	9.4	9.6		1 mo.: 0.9
	2	N_2	5.2	9.0	3.0	8.0	2.7	6.6	3 mo.: 0.6
	2	air	4.2	7.8	2.9	6.0	1.9	3.9	6 mo.: 0.7
	5	N_2	3.6	7.7	2.4	6.1	....	....	
	5	air	2.7	5.8	1.3	4.4	....	....	
			5 wks.						5 wks.: 1.1
12A. Spray-dried powders. Fermentation with commercial bakers' yeast (*S. cerevisiae*)	(Control)	.....	9.6	9.7	....	....	....	....	
	5	N_2	2.8	7.4	....	....	....	....	
	5	air	2.6	5.1	....	....	....	....	
					3 mo.				
17. Lyophilized powders. Fermentation with laboratory-grown *S. cerevisiae* 2040	2	N_2	....	....	6.0	9.4	....	....	3 mo.: 1.0
	2	air	....	....	4.3	7.4	....	....	

[b] Glucose-free powders prepared in the laboratory by yeast fermentation aand lyophilization retained a slight residual "yeastiness" which was detected by some members of the trained taste panel. That this did not interfere with the detection of storage off-flavor was shown by a preliminary tasting experiment in which very slight degrees of storage off-flavor (artificially produced by admixing small amounts of stored egg powder with freshly lyophilized powder) were found to be just as readily detected in the yeast-fermented as in the untreated powders. The spray-dried glucose-free powders, which were prepared in a commercial plant, did not pose a problem in this regard, as residual yeastiness was not detectable to a significant degree.

[c] U represents untreated (glucose-containing) powders. F represents fermented (glucose-free) powders.

[d] Control samples were prepared from the same batch of liquid egg and kept at —34° C. (—30° F.).

Effect of Glucose Removal on Flavor Stability. The data in Table 1 show that palatability retention at 37.5° C. (100° F.) is much greater for glucose-free than for ordinary glucose-containing dried egg. This is true for lyophilized and spray-dried powders, for 2% and 5% moisture powders, and for nitrogen- and air-packed samples. As will be shown later it is also true under certain conditions for storage at 15.5° C. (60° F.) as well as at 37.5° C. (100° F.).

The following points are of special interest: (a) The magnitude of the stabilizing effect of glucose removal is large under all storage conditions tested. (b) The stabilizing effect is consistently obtained in independent experiments. (c) Attainment of flavor stability at 37.5° C. (100° F.), even under the most optimal conditions studied (2% H_2O, N_2 pack), appears to be impossible without retardation of glucose-induced reactions. (d) Flavor deteriorations are minimal in the low-moisture (2%), nitrogen-packed, glucose-free powders. Such powders (see Experiments 1, 6, and 17 in Table 1) suffered little significant flavor damage even after two to three months at 37.5° C. (100° F.). (e) While the contributions of the non-glucose-type reactions to flavor instability appear to be of relatively little importance in the low-moisture, nitrogen-packed, glucose-free powders, under other storage conditions non-glucose-type reactions are important. Storage in the presence of air is accompanied by off-flavor development in both the low-and high-moisture glucose-free powders, while storage of nitrogen-packed glucose-free powders at 5% moisture causes non-oxidative reactions that do not occur significantly in nitrogen-packed glucose-free powders at 2% moisture.

Effect of Glucose Removal on "Browning" and the Relation of "Browning" to Off-Flavor. "Browning" as used here will refer to the visible color changes from yellow to brown that occur during storage, and that are apparent in the final "scrambled egg" product. Although the extent of browning was not quantitatively measured, some of the observations were so striking as to warrant a qualitative report.

As pointed out by Dutton and Edwards *(3)*, color changes in whole-egg powder during storage may represent the net effect of several simultaneous reactions, such as (a) the oxidative destruction of the carotenoid pigments, which are responsible in large part for the natural color of egg, and (b) the nonenzymatic browning reactions. The significance of the present results with regard to the role of glucose in these latter reactions may be summarized in the following observations:

(a) Removal of glucose essentially eliminated the development of brown substances. "Scrambled eggs" prepared from glucose-free powders which had been stored 6 months at 37.5° C. (100° F.) showed little, if any, significant browning (Figure 1). Of course, glucose removal did not prevent the decrease in the natural yellow color due to oxidative destruction of the carotenoids, as may be readily deduced from the lipid absorption measurement in the region of 445 mμ (Figure 3).

(b) For the untreated (glucose-containing) powders, the browning was consistently observed to be much more pronounced in the nitrogen-packed powders than in the air-packed powders (Figure 1). This effect was obvious at both 2 and 5% moisture. The greater degree of browning under nitrogen pack was so pronounced that it is improbable that it resulted from a combination of carotenoid retention plus the same amount of browning that occurred in the air-packed powders.

(c) Most of the chemical measures of the nonenzymatic browning reactions used in the present work were not reliable indicators of the degree of browning exhibited by the stored untreated powders. In particular, these measurements failed to reflect consistently the many times greater browning visible in the nitrogen-packed untreated powders. Thus the data obtained for salt-water-soluble fluorescence, ether-soluble fluorescence, and lipid ultraviolet absorption were not always correlated with the differences in browning actually observed (Table 2 and Figure 1). One set of objective measurements which, however,

TABLE 2

Effect of glucose removal on chemical changes in dried whole eggs stored at 37.5° C. (100° F.)

Sample		Glucose in powder, %		pH		Protein solubility, %		Salt-water-soluble fluorescence, Φ		Ether-soluble fluorescence, Φ		Absorbancy of phospholipid fraction (redissolved in ether)			
Moisture content, %	Stored in											10 mg./ml., at 500 mμ		5 mg./ml., at 300 mμ	
		U[a]	F[a]	U	F	U	F	U	F	U	F	U	F	U	F
Experiment 6, lyophilized powders stored 1 month															
(Control)[f]	....	1.10	0.03	8.6	8.7	96	94	1.0	1.6	1.2	0.9	0.02	0.03	0.26	0.26
2	N_2	0.42	.....	8.4	8.7	83	91	5.1	1.6	15.5	1.8	0.17	0.03	1.04	0.35
2	air	0.41	.....	8.3	8.6	80	91	5.1	1.9	14.2	2.4	0.09	0.04	1.11	0.46
5	N_2	0.05	.....	8.1	8.6	66	87	18.0	2.5	18.0	1.6	0.20	0.03	1.04	0.27
5	air	0.06	.....	7.8	8.6	60	87	9.4	2.6	16.2	2.0	0.10	0.03	0.93	0.31
Experiment 6, stored 3 months															
(Control)	....	1.12	0.03	8.8	8.8	96	93	2.2	1.6	1.0	1.0	0.02	0.02	0.20	0.23
2	N_2	0.29	.....	8.1	8.7	74	94	11.4	2.9	26.8	2.5	0.26	0.03	1.77	0.30
2	air	0.25	.....	7.8	8.6	66	89	9.6	3.8	22.8	4.3	0.11	0.04	1.74	0.54
5	N_2	0.04	.....	7.5	8.4	49	89	26.0	4.5	27.5	2.0	0.33	0.03	1.50	0.22
5	air	0.04	.....	7.1	8.3	45	86	14.4	4.9	23.0	2.8	0.12	0.02	1.46	0.34
Experiment 12A, spray-dried powders stored 5 weeks															
(Control)	....	1.07	0.03	8.6	8.8	85	89	1.6	1.3	0.7	0.6	0.02	0.02	0.25	0.32
5	N_2	0.06	.....	7.7	8.6	60	85	18.0	3.7	19.0	2.5	0.23	0.03	1.01	0.34
5	air	0.06	.....	7.5	8.5	58	79	15.5	4.0	17.0	3.3	0.16	0.03	1.00	0.41

[a] U represents untreated (glucose-containing) powders. F represents fermented (glucose-free) powders. [f] Control samples were prepared from the same batch of liquid egg and kept at —34° C. (—30° F.).

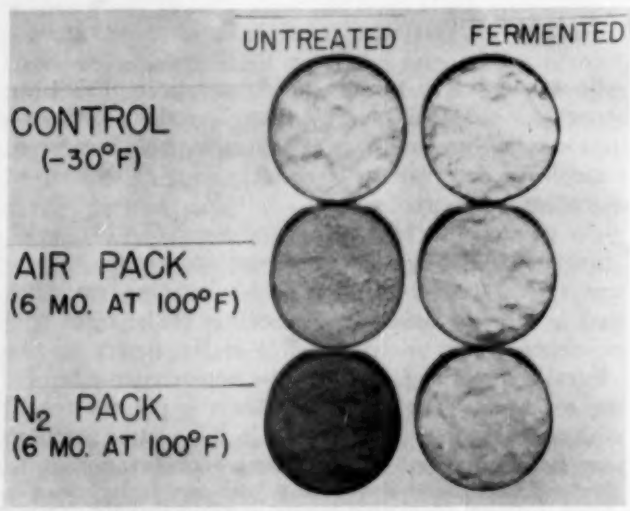


FIG. 1. Effect of glucose removal on elimination of browning during storage. The "scrambled eggs" illustrated were prepared from the 5% moisture powders of Experiment 6 (see Tables 1, 2).

gave data that did consistently reflect the greater browning in the nitrogen-packed powders was the absorbency measurements on the isolated phospholipids in the visible part of the spectrum (Table 2, 500 mμ). These measurements also reflected the much more intense browning visibily apparent in the phospholipid fractions isolated from powders stored in a nitrogen atmosphere.

While it appears certain that the phenomenon of greater browning in the whole powders stored in nitrogen is, in part, attributable to changes in the phospholipid fraction, the contribution of the browning in the fat-free fraction cannot be excluded on the basis of the present data. The salt-water-soluble fluorescence, which serves as a measure of the soluble brown products in the fat-free fraction, did, in certain cases (Table 2), suggest greater browning in fractions isolated from nitrogen-packed powders. However, this measure does not include the contribution of that part of the fat-free fraction that became insoluble during storage. It is probable that reflectance spectra measurements on the total fat-free fraction would yield a truer picture of the contribution this fraction makes to the phenomenon of greater browning under nitrogen *(3)*.

(d) There may be a correlation between degree of browning and degree of off-flavor for comparisons involving only nitrogen-packed untreated powders, since the browning reactions are the predominant causes of off-flavor in such cases. However, for air-packed untreated powders the degree of browning appears to be a poor indicator of off-flavor development. Such samples consistently showed more pronounced off-flavors but less browning than their counterparts stored in nitrogen (Tables 1 and 2). Also, some of the glucose-free powders, which showed little or no evidence of browning, developed pronounced off-flavors on extended storage (Table 1).

Effect of Glucose Removal on Baking Quality Retention. The baking qualities of the untreated powders deteriorate rapidly during storage at 37.5° C. (100° F.), particularly in the case of the 5 percent-moisture powders (Figure 2). At this moisture level, after three months of storage, these powders had lost so much of their whipping ability that the specific gravities of their meringues were close to 1.0 and the sponge-cake volumes approached an irreducible minimum. In contrast, the baking quality of the glucose-free samples after storage were so markedly superior to those of glucose-containing powders that there is little doubt that loss of baking quality during high-temperature storage is due to glucose-induced reactions.

For the untreated powders, reducing the moisture content from 5 to 2% was much more effective in retarding the loss in baking function than it was in retarding off-flavor development (Table 1). This apparent anomaly is interpretable in terms of the effect of moisture content on certain chemical reactions as indicated in the analyses reported in Table 2. These analyses show that moisture reduction sharply retards the glucose-protein interaction but retards only slightly, if at all, the interaction of glucose and cephalin. Thus, the glucose-protein interaction appears to be a primary cause of deterioration in baking quality, while the glucose-cephalin interaction manifests itself more directly in the depreciation in flavor quality.

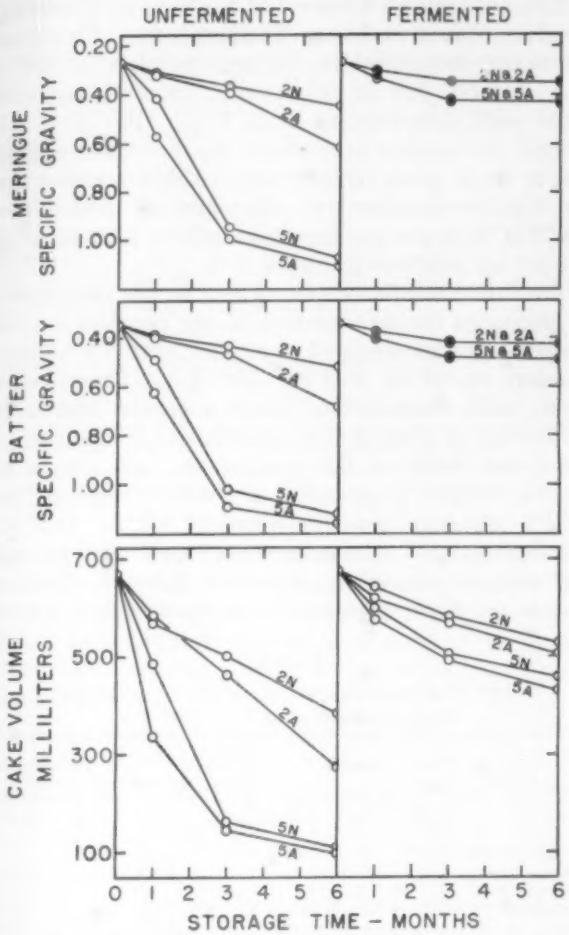


FIG. 2. Effect of glucose removal on retention of baking quality during storage at 37.5° C. (100° F.). Sponge cake data for Experiment 6 illustrated. Descriptions indicate moisture percentage of powder and gas pack during storage, that is, 2N represents 2% moisture powder stored in nitrogen; 2A represents 2% moisture powder stored in air.

Effect of Glucose Removal on Chemical Changes During Storage. In attempting to understand why glucose removal effects such substantial improvements in organoleptic and functional stabilities, it is worthwhile to examine its effect on the chemical changes that normally occur during the storage of the untreated egg powders. Some of these are illustrated in Table 2 and Figure 3. Results for spray-dried powders are again included to show that comparable effects are obtained with these powders.

The reactivity of glucose may be judged from the rapidity with which it disappears during storage. The rate of loss is greater at the higher (5%) moisture level, in which case it is essentially gone after 1 month of storage.

The extent of the glucose-protein interaction may be esttimated from changes in protein solubility, salt-water-soluble fluorescence, and pH. The first two measurements are clearly associated with changes in the protein constituents, while the pH changes measured on the fat-free residues (mainly protein) have been found to be nearly identical with the pH changes measured on the whole-egg powder. Glucose removal eliminates or greatly reduces the magnitude of all three changes during storage. It is evident from these measurements (Table 2) that the glucose-protein interaction in the untreated powder occurs much more extensively at the higher moisture level. As already noted, the retention of baking performance varied in a similar manner. It seems highly probable that the excellent retention of baking performance in the glucose-free powders is attributable to the elimination of the glucose-protein interaction. In this regard the present results are in close agreement with the previously reported observations of the British investigators *(8)*. These investigators, however, evaluated the effect of glucose removal only on foaming volume.

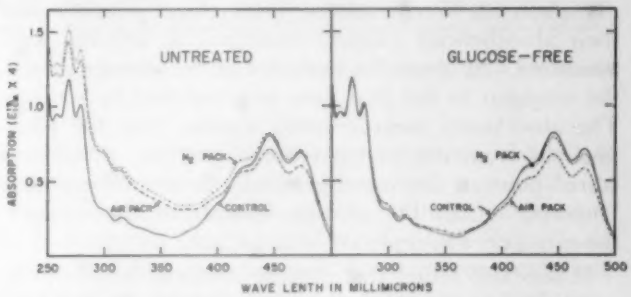


FIG. 3. Effect of glucose removal on storage-induced changes in absorption spectra of ether extracts. The two samples illustrated were spray-dried powders stored 5 weeks at 37.5° C. (100° F.). (Expt. 12A).

A recent report by some of the present authors showed that certain changes in the lipid constituents during storage of egg powder were a measure of the glucose-cephalin interaction *(10)*. These included increases in fluorescence and ultra-violet absorption as measured either on the total ether extract or on the phospholipid fraction prepared therefrom, as well as the increase in absorption of the phospholipids in the visible portion of the spectrum. It is clear from the data in Table 2 and Figure 3 that these changes are essentially eliminated by glucose removal. In contrast to the effect of the moisture content of the powder on the glucose-protein interaction, the glucose-cephalin interaction apparently proceeded as extensively in the 2% moisture untreated powders as in the 5% moisture powders.

Effect of Glucose Removal on the Uptake of Oxygen and the Liberation of Carbon Dioxide During Storage. It has been tacitly assumed that oxygen uptake by egg powders, as for other lipid-rich foodstuffs, is attributable to autoxidative deterioration of the lipids. Hence, it was somewhat surprising to find that the oxygen uptake by egg powders was reduced two- to three-fold by glucose removal before drying. Illustrative data for both lyophilized and spray-dried powders and for storage at 15.5 and 37.5° C. (60 and 100° F.) are given in Table 3.

These results show that oxygen uptake by ordinary (glucose-containing) egg powders is markedly greater than that by the comparable glucose-free powders. Of course, it is not implied that oxygen is necessary for the amine-aldehyde browning reactions; the opposite has been shown to be true in the present report. Also, the occurrence of nonenzymic browning reactions in other concentrated foodstuffs is known to be largely inde-

pendent of oxidative action. In the case of the untreated egg powders there is probably a connection between the increased oxygen uptake accompanying the glucose-type reactions and the lesser degree of visible browning found in these powders stored in air (as compared to those stored in nitrogen). It is conceivable that a reaction occurs between oxygen and the "browned" secondary products of reactions involving glucose, which results in a bleaching action. Dr. Makower of this laboratory has recently pointed out to the senior author that some unpublished results obtained by him in 1945 tend to support this hypothesis. He stored whole-egg powder in both nitrogen and oxygen at 49° C. (120° F.) for about 4 to 5 months. Periodically the phospholipids were isolated from these powders and their absorbencies (optical densities) at 440 mμ were measured. At about the midpoint of the storage period, the nitrogen in the first case was replaced by oxygen. The absorbency measurements showed that the phospholipid from the nitrogen-stored powder, which was stored powder, decreased gradually in absorbency after "browner" than the phospholipid from the oxygen-the nitrogen was replaced with oxygen.

It was also found that glucose removal definitely decreased the amount of carbon dioxide evolved during storage (Table 3). This was true for both nitrogen-packed and air-packed powders. There is little doubt, therefore, that part of the carbon dioxide evolved results from glucose interactions although not necessarily from the glucose per se. Further work is required to establish the origin of this carbon dioxide. Also noteworthy was the finding that carbon dioxide evolution from both the ordinary and the glucose-free powders was markedly greater when these powders were stored in air than when stored in nitrogen.

TABLE 3

Effect of glucose removal on changes in the gas phase during storage [a]

Experiment no.	Sample		Gas analyses [b]		
			Air-packed		N_2 packed
	Moisture content, %	Type of egg [b]	O_2 decrease, vol. %	CO_2 increase, vol. %	CO_2 increase, vol. %
12A. Spray-dried powders. Storage 5 wks. at 100° F.	5	U	20.7	4.6	1.5
	5	F	10.0	1.0	0.2
17. Lyophilized powders. Storage 3 mo. at 100° F.	2	U	20.5	13.3	4.1
	2	F	9.6	5.8	3.3
12B. Spray-dried powders. Storage 5 mo. at 60° F.	5	U	15.6	1.3	0.2
	5	F	4.5	0.2	0.0
Storage 10 mo. at 60° F.	5	U	20.8	2.2	0.6
	5	F	7.7	0.8	0.3

[a] All samples: Approx. 8 oz. powder in No. 2 can. For the meaning of the letters U and F, see footnote [c], Table 2.

[b] Changes in volume percent are reported. The gas in the air-packed samples analyzed originally 20.8 vol. percent O_2 and 0.0 vol. percent CO_2. Thus a decrease in vol. percent of O_2 during storage of 20.8 represents complete absorption of the O_2. The gas in the N_2-packed samples analyzed originally 0.0 vol. percent O_2 and 0.0 vol. percent CO_2.

Effect of Glucose Removal on Stability of Whole-Egg Powders Stored at Lower Temperatures. Flavor and functional deteriorations in egg powders stored at 15.5° C. (60° F.) or below occur quite slowly, compared with deterioration at 37.5° C. (100° F.). The meager information available in the literature suggests that at these lower temperatures oxidative rather than the high-temperature-type deterioration predominates and that inert-gas packing alone suffices to stabilize the powder for practical purposes.

The limited studies reported here on the participation of glucose in the deterioration of egg powders at lower temperatures are confined to evaluation of 5% moisture powders stored at 15.5° C. (60° F.). These experiments were conducted to obtain a general idea of the occurrence of glucose-type reactions at lower temperatures and therefore the conclusions may not be applicable without qualification to specific conditions, such as 2% moisture powders stored at 1.5° C. (35° F.). The retention of palatability as a function of gas pack and glucose removal is shown in Table 4. Data are shown for both lyophilized and spray-dried powders stored under identical conditions. The two sets of powders were, however, prepared from different parent melanges; the lyophilized powders from California eggs and the spray-dried powders from eggs produced in the Midwest. In general the data show that under certain conditions the glucose-type reactions contribute in an important way to flavor deterioration during storage at 15.5° C. (60° F.) The results for each set of powders will be considered separately, since it was found that, unlike the results for storage at 37.5° C. (100° F.), the lyophilized powders were significantly more stable than comparable spray-dried samples.

TABLE 4

Effect of glucose removal on off-flavor development during storage at 15.5° C. (60° F.) [a]

Experiment no.	Sample		Palatability scores				Difference required for significance at 5% level
			Storage time at 60° F.				
	Moisture content, %	Stored in	3 mo.		10 mo.		
			U	F	U	F	
10. Lyophilized powders. Fermentation with *T. monosa*	Control	....	9.9	9.7	9.8	9.7	3 mo.: 0.5
	5	N_2	9.7	9.7	8.4	8.9	10 mo.: 0.9
	5	air	8.7	9.5	6.0	8.0	
			5 mo.		10 mo.		
12B. Spray-dried powders. Fermentation with commercial bakers' yeast	Control	....	8.5	9.2	9.3	9.3	5 mo.: 0.8
	5	N_2	5.9	8.7	5.1	8.0	10 mo.: 1.1
	5	air	4.1	6.2	3.7	5.5	

[a] For the meaning of the letters U and F see footnote [c] Table 2.

In the case of the lyophilized powders, nitrogen packing alone was found to result in adequate stabilization. Thus after 10 months at 15.5° C. (60° F.), the palatability score assigned the untreated nitrogen-packed powder (8.4) indicated that the off-flavor development was barely significant. This was true also for the nitrogen-packed glucose-free powder (palatability score, 8.9). Comparison of the lyophilized powders packed in air showed, however, that glucose removal gave a marked improvement in flavor stability. The greater degree of off-flavor in the untreated air-packed powder was consistent with the gas analyses results (Table 5),

TABLE 5

Effect of glucose removal on chemical changes in dried whole eggs stored 10 months at 15.5° C. (60° F.)

Sample		Glucose in powder, %		pH		Protein solubility, %		Salt-water soluble fluorescence, Φ		Ether-soluble fluorescence, Φ		Absorbancy of phospholipid fraction in ether				Gas analyses [k]			
												10 mg./ml., at 500 mμ		5 mg./ml., at 300 mμ		CO_2, vol. %		O_2, Vol. %	
Moisture content	Stored in	U	F[j]	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F
Experiment 10, lyophilized powders																			
(Control)[k]	....	1.08	0.02	8.8	8.8	92	93	2.0	2.0	0.5	0.5	0.01	0.01	0.15	0.15	0.0	0.0	0.0	0.0
5	N_2	0.45	.....	8.5	8.8	89	92	6.6	2.0	4.5	0.8	0.05	0.02	0.35	0.19	0.4	0.0	0.0	0.0
5	air	0.32	.....	8.4	8.7	89	90	7.0	2.3	4.8	1.2	0.03	0.02	0.43	0.24	2.8	0.8	13.1	17.3
Experiment 12B, spray-dried powders																			
(Control)	....	1.07	0.03	8.6	8.8	86	86	1.8	1.8	0.8	0.7	0.01	0.01	0.18	0.21	0.0	0.0	0.0	0.0
5	N_2	0.26	.....	8.1	8.6	73	83	9.0	2.5	11.0	2.1	0.08	0.01	0.51	0.26	0.3	0.0	0.0	0.0
5	air	0.26	.....	8.0	8.5	71	81	6.5	3.2	9.7	3.3	0.05	0.01	0.58	0.31	2.2	0.8	0.0	13.1

[j] In Experiment 10 the glucose-free powders were prepared by fermentation with *T. monosa*. In Experiment 12B commercial bakers' yeast was used. For the meaning of the letters U and F see footnote [e], Table 2.

[k] Control samples were N_2-packed and kept at —34° C. (—30° F.). Gas in N_2-packed samples analyzed originally as 0.0 vol. percent O_2, 0.0. vol. percent CO_2; air-packed samples as 20.8 vol. percent O_2, 0.0 vol. percent CO_2.

which showed that the untreated air-packed powder absorbed nearly twice the amount of oxygen absorbed by the comparable glucose-free powder. These results suggest that the participation of glucose in off-flavor development includes a contribution to oxidative-type flavor damage.

Results obtained with the spray-dried powders differed in important respects from those obtained with the lyophilized set. First, nitrogen packing alone did not prevent pronounced off-flavor development in the untreated spray-dried powder under the identical storage conditions in which it protected the lyophilized powder. Secondly, although the glucose-free air-packed powder did not develop as much off-flavor as did the untreated powder, in both cases the extent of the flavor deterioration in the spray-dried air-packed powders was much greater than that occurring in the comparable lyophilized samples stored identically. In brief, then, it was found that nitrogen packing alone was an effective stabilization means for the lyophilized powders stored at 15.5° C. (60° F.), but that both nitrogen packing and glucose removal were necessary to stabilize the spray-dried powders.

The explanation for the greater stability of the set of lyophilized powders may lie in certain basic differences between the two types of powders as follows: (a) Difference in composition. One known difference was in carotenoid content. Spectrophotometric analysis showed that the spray-dried powders contained nearly three times as high a concentration as the lyophilized powders. Whether the carotenoids contribute in a significant way to oxidative flavor damage has not, to our knowledge, been investigated. (b) Differences in particle structure. Spray-dried powders are known to be composed of hollow, more or less spheroidal particles and to contain coalesced fat globules, while the lyophilized powders consist of thin platelets, and fat coalescence is minor *(13)*. (c) Initiation of deteriorative reactions during spray-drying. It is possible that both the Maillard-type reactions involving glucose, and the oxidative lipid deteriorations are initiated during spray-drying. Evidence for the former was found in the analyses for glucose (Somogyi method) which revealed that about 25% of the glucose contained in the parent melange was not determinable after spray-drying.

The chemical analyses of the two sets of powders after storage for 10 months at 60° C. (15.5° F.) (Table 5) showed that the glucose-induced protein and lipid deteriorations were indeed more extensive in the spray-dried powders. The gas analyses results make it evident also that oxygen uptake by the spray-dried powders was much greater.

At present it is not possible to decide whether the more extensive chemical changes in the spray-dried powders were due to initiation of reactions during spray-drying or to a greater rate of change during storage due to differences in chemical composition or physical structure.

DISCUSSION

The results leave no reasonable doubt that the glucose in dried whole egg leads to serious flavor and baking quality deteriorations. The mechanism by which glucose exerts its deleterious effect is explainable in terms of the glucose-cephalin and glucose-protein interactions. In the latter case the term applies to the reaction of the glucose with the proteins of both the yolk and white and should not therefore be misconstrued as referring only to the classical glucose-albumen reaction.

First, with regard to baking quality, it seems fairly certain that retention of this function is dependent upon elimination of the glucose-protein reactions. Loss of baking quality in the normal glucose-containing powders under various storage conditions was found to correlate well with the chemical measures of the glucose-protein reactions, such as salt-water-soluble flourescence and protein solubility. Also, of course, removal of glucose before drying yielded powders which retained their baking quality over extended storage periods.

Regarding the manner in which glucose induces the development of off-flavors, there is considerable evidence which suggests that the glucose-cephalin reaction plays a predominant role. The report by Fevold, Edwards, Dimick, and Boggs *(5)* showed that egg white, dried and then stored separately from the yolk at elevated temperatures, developed considerable salt-water-soluble fluorescence, but made very little contri-

bution to off-flavor when it was mixed with freshly dried yolk in the proportion of whole-egg powder and evaluated as "scrambled eggs." On the other hand, dried yolk or certain fractions therefrom, when stored separately at elevated temperatures, yielded the typical storage off-flavors found in dried whole-egg stored in a similar manner. Additional evidence was provided by Boggs, Dutton, Edwards, and Fevold *(1)*, who examined numerous stored dried whole-egg samples, and showed that salt-water-soluble fluorescence was, in many cases, a poor index of flavor deterioration, whereas an excellent correlation was found between fat-soluble-fluorescence and flavor deterioration. These results suggested the lipids as the major source of off-flavors and numerous fractionation studies at this laboratory on stored egg powders have shown that the brown pigments, off-odors, and in a qualitative way, off-flavors, are situated almost entirely in the phospholipid fraction. Thus, the identification of the phospholipids as the principal site of off-odor and off-flavor development coupled with the demonstration by some of the present authors that the most rapid reaction in dried whole-egg powders leading to phospholipid deterioration results from the interaction of glucose and cephalin *(10)* presents strong evidence for the predominant mechanism of glucose-induced flavor deterioration. These considerations do not, of course, eliminate the glucose-protein reactions as participants in off-flavor development, but appear to relegate them to a minor role.

As pointed out in the discussion of off-flavor development as a function of storage conditions, the removal of glucose, by itself, is insufficient to effect complete stabilization. Flavor deteriorations in the glucose-free powders occurred during storage in air or at high moisture levels. Little is known of the specific chemical changes responsible for these non-glucose-type flavor deteriorations, although the instability of the phospholipids to autoxidation causes these substances to be suspected as the site of oxidative flavor damage. The usual tests such as peroxide value, iodine number, and Kreis value were made on the various lipid fractions in the present work and were found to be negative or to indicate such slight changes as to be useless as an index of oxidative flavor deterioration. Carotenoid measurements on powders of unknown original composition are difficult to interpret, since the initial content of egg in these substances is quite variable. A sensitive chemical measure of oxidative flavor damage would be very useful, particularly when used in conjunction with the chemical indicators of the glucose-type reactions. Interpretation of the flavor deterioration induced by storage at higher moisture levels must also await further knowledge, although some changes have been found to occur to a much greater extent in the high-moisture samples. These include increases in free fatty acids (measured on the acetone-soluble fraction of the ether extract), loss in free amino groups in the phospholipid fraction, and slow insolubilization of protein.

Some Practical Implications. It has been shown that the free glucose in whole-egg powder, which is present in amounts slightly over 1% of the total solids, induces the principal part of the quality deterioration during storage at 37.5° C. (100° F.). From a practical standpoint, the removal of the glucose is a feasible means of increasing the stability of whole-egg powder. It should be noted too that the nutritive value of the egg powder is not significantly impaired by the removal of approximately 1% carbohydrate (dry basis). It has proved convenient in the present work to use a resting-cell yeast fermentation procedure to effect the glucose removal, since preliminary studies showed that an organoleptically acceptable glucose-free powder could be prepared by this method *(9)*. However, there is no a priori reason why other procedures designed to "inactivate" the glucose might prove equally or more satisfactory. For example, conversion of glucose to gluconic acid by the use of purified glucose oxidase preparations from mold [1] may prove advantageous and is presently under investigation in this laboratory. The pre-acidification process for stabilizing whole-egg powder, which is currently specified by the Quartermaster procurement authorities *(11)*, is effective because it retards the glucose-induced reactions, apparently without retarding non-glucose-type deteriorations (a manuscript containing this evidence is in preparation at this Laboratory). Further work is needed to determine which process is the most suitable from the standpoints of reproducibility in commercial practice, economy of process, initial quality, and stability of product. Obviously, one process might be preferred for some purposes and another process for other purposes.

CONCLUSIONS

1. Flavor stability of whole-egg powder stored at 37.5° C. (100° F.) has been shown to be primarily dependent upon elimination of glucose-induced reactions. Non-glucose-type deteriorations, both oxidative and non-oxidative, were readily demonstrated by the use of glucose-free powders, but these deteriorations were neither as rapid nor as marked as the glucose-type deteriorations. A combination of glucose removal, low moisture, and inert-gas packing yielded a product of marked stability, which showed little significant flavor deterioration even after storage for two to three months at 37.5° C. (100° F.).

2. Baking quality stability, as assessed by a high-temperature-whip sponge cake test, was also largely dependent upon elimination of the glucose-induced deteriorations, particularly glucose-protein type deteriorations. Increased stability due to glucose removal was more striking at the 5% moisture level than at lower moistures. Thus, powders that contained glucose failed to whip appreciably after 3 months at 37.5° C. (100° F.), whereas the glucose-free powders still performed excellently after 6 months of storage.

3. There are certain pronounced physical and chemical changes that occur during storage of normal (glucose-containing) egg powders which are either

[1] Such an enzyme is available from Vita-zyme Laboratories, Inc., Chicago, Ill.

eliminated or greatly reduced by glucose removal. These include the following: visible browning, pH decrease, increase in salt-soluble fluorescence, increase in lipid fluorescence, increase in ultraviolet absorption by lipid constituents, and decrease in protein solubility. Additionally, it was found that the oxygen uptake by air-packed powders was reduced two- to three-fold by glucose removal. These changes have been shown to be in large part manifestations of the glucose-protein and glucose-cephalin interactions. The glucose-protein interaction was sharply decreased when the moisture content of the powder was reduced from 5 to 2% but the glucose-cephalin interaction was not.

The mechanism of oxidative deterioration in egg powder remains to be elucidated and the relation of glucose to oxidative flavor damage is not clear. The lack of a sensitive chemical test for the non-glucose-type of oxidative deterioration in quality is now perhaps the most serious deterrent to the successful objective evaluation of the quality of egg powders of unknown history by means of interpreting chemical tests in place of the more difficult and expensive organoleptic methods.

4. Browning, as apparent to the eye, was essentially eliminated by glucose removal. Since both the glucose-protein and glucose-cephalin interactions lead to the formation of brown substances, the explanation for the beneficial effect is clear. Other types of nonenzymic browning do not appear to be important.

Oxygen influenced the browning in glucose-containing dried egg. Thus, untreated powders brown much more intensively when stored in a nitrogen atmosphere than in air. This correlated directly with the appearance of brown color in the isolated phospholipid fractions and to a certain extent with the increase in salt-soluble fluorescence of the fat-free residues.

The degree of browning did not necessarily correlate with degree of off-flavor. Thus, in all cases the untreated powders stored in air scored lower for flavor than their counterparts stored in nitrogen, although the latter samples were invariably "browner."

5. Under certain conditions the glucose-type reactions were found to play an important role in flavor deterioration in egg powders stored at 15.5° C. (60° F.). Lyophilized powders were more stable at this temperature than comparable spray-dried powders. Nitrogen packing alone was sufficient to prevent appreciable flavor deterioration in lyophilized powders stored 10 months at 15.5° C. (60° F.), but both nitrogen packing and glucose removal before drying were necessary to stabilize comparable spray-dried powders stored under identical conditions.

Acknowledgment and Correction

The authors thank Stanley Friedlander and Glen Bailey for the spectrophotometric measurements and are greatly indebted to numerous Western Regional Research Laboratory employees who served as judges in the organoleptic evaluations and to Kazuko Nishita, who assisted in both the taste panel work and the sponge-cake preparations.

A previous article in this series *(10)* states (on pages 181-2) that "Edwards and Dutton fractionated the 'browned' phospholipid obtained from whole-egg powder . . . into crude cephalin and lecithin and found that the pigment accompanied the cephalin fraction." As explained by Edwards and Dutton this work was actually done by E. B. Kester of Western Regional Research Laboratory.

LITERATURE CITED

1. Boggs, M. M., Dutton, H. J., Edwards, B. G., and Fevold, H. L. Dehydrated egg powders. Relation of lipid and salt-water fluorescence values to palatability. *Ind. Eng. Chem.*, **38**, 1082 (1946).
2. Dutton, H. J., and Edwards, B. G. Changes in stored dried eggs. Spectrophotometric and fluorometric measurements of changes in lipids. *Ind. Eng. Chem.*, **37**, 1123 (1945).
3. Dutton, H. J., and Edwards, B. G. Changes in color of dehydrated eggs during storage. *Ind. Eng. Chem.*, **38**, 347 (1946).
4. Edwards, B. G., and Dutton, H. J. Changes in stored dried eggs. Role of phospholipids and aldehydes in discoloration. *Ind. Eng. Chem.*, **37**, 1121 (1945).
5. Fevold, H. L., Edwards, B. G., Dimick, A. L., and Boggs, M. M. Dehydrated egg powders. Sources of off-flavors developed during storage. *Ind. Eng. Chem.*, **38**, 1079 (1946).
6. Hanson, H. L., Kline, L., and Lineweaver, H. Application of balanced incomplete block design to scoring of ten dried egg samples. *Food Technol.*, **5**, 9 (1951).
7. Hawthorne, J. R. Solubility in potassium chloride. L. T. R. S. method. *J. Soc. Chem. Ind.* (London), **63**, 6 (1944).
8. Hawthorne, J. R., and Brooks, J. Removal of the sugar of egg pulp before drying—method of improving the storage life of spray-dried whole egg. *J. Soc. Chem. Ind.* (London), **63**, 232 (1944).
9. Kline, L., and Sonoda, T. T. Role of glucose in the storage deterioration of whole egg powder. I. Removal of glucose from whole egg melange by yeast fermentation before drying. *Food Technol.*, **5**, 90 (1951).
10. Kline, L., Gegg, J. E., and Sonoda, T. T. Role of glucose in the storage deterioration of whole egg powder. II. A browning reaction in dried whole eggs involving glucose and cephalin. *Food Technol.*, **5**, 181 (1951).
11. Military specification. Stabilized dehydrated egg. QMC Mil-e-10006, (Sept. 12, 1949).
12. Pearce, J. A., Thistle, M. W., and Reid, M. Improved fluorescence method. *Can. J. Research*, **21D**, 341 (1943).
13. Reeve, R. M. Unpublished results, Western Regional Research Laboratory (1944).
14. Somogyi, M. Determination of blood sugar. *J. Biol. Chem.*, **160**, 69 (1945).
15. Western Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture. Information sheet on the sampling and analysis of gases in cans of dehydrated vegetables. (June, 1944).

A New Graphical Interpolation Method for Obtaining Humidity Equilibria Data, with Special Reference to Its Role in Food Packaging Studies[a]

ARTHUR H. LANDROCK AND BERNARD E. PROCTOR
Department of Food Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts

(Manuscript received May 5, 1951)

A method is described for determining equilibrium relative humidities of a wide variety of products rapidly and with simple equipment. All factors involved in the determination are held constant except relative humidity. Samples of a product of a certain moisture content are exposed to different relative humidities for an arbitrary constant time, usually one hour. Each sample is then weighed once only, in contrast to the usual methods whereby repeated weighings are made until weight equilibrium is reached. The gains and losses in weight per unit of time are plotted with reference to relative humidity. By interpolation, the equilibrium relative humidity is read at that point where the smooth curve drawn through the plotted data intersects the baseline representing no weight change. Repetition of this procedure with other samples of other moisture contents gives data for a humidity-moisture equilibrium curve covering a wide range of relative humidities and equilibrium moisture contents. Approximately two days are necessary to secure this curve.

To package any food product most efficiently, the packaging technologist must first know the properties of that product and the properties of any packaging materials he might use. There are numerous tests for measuring the properties of packaging materials *(2)*, and many of these are in routine use throughout the country. Relatively little is known, however, about the properties of food products that must be considered in designing the functional aspects of packages, in other words, about the product requirements and the product evaluation.

From the packaging point of view, hygroscopicity is one of the most important characteristics of dry foods. In general, hygroscopicity simply means the tendency of the product to attract water vapor from the atmosphere. But moisture losses from moist products are equally important in packaging. Unfortunately there is no one word in English usage applicable to such moist products that means "tending to give off water vapor." The authors therefore suggest a new word having exactly the opposite meaning of hygroscopic. This word is "hygro-emissive."

Obviously the moisture content of a food product will have a strong bearing on whether the product is hygroscopic or hygro-emissive and to what degree. The most common means of expressing this relationship is the humidity-moisture equilibrium curve, as illustrated in Figure 1. Curves of this type show throughout their entire length an equilibrium relationship between the moisture content of the product and the relative humidity of the atmosphere immediately over the product.

Examination of the curve for plain whole soda crackers in Figure 1 shows that the crackers as packaged, that is, with a moisture content of 4.48%, were in equilibrium with a relative humidity of 15.5%. This means that the crackers neither gained nor lost moisture at this relative humidity. Under such conditions the relative humidity is termed the "equilibrium relative humidity" or "ERH."

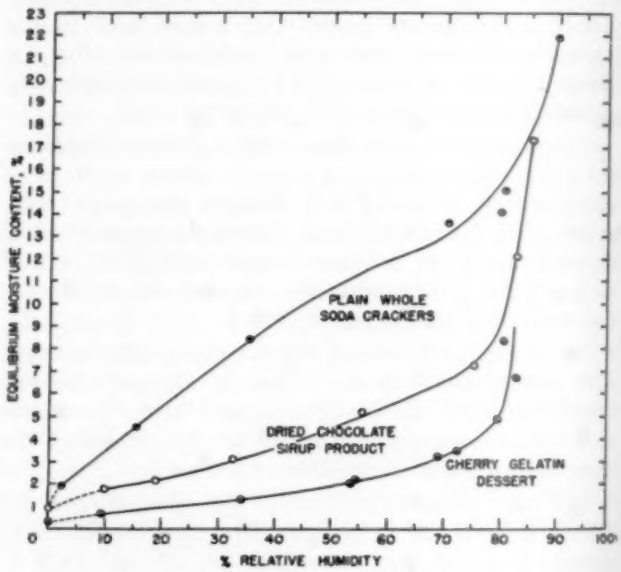


FIG. 1. Humidity-moisture equilibrium curves for various food products, as obtained by the graphical interpolation method (25° C.).

It is readily apparent that these crackers will undergo desorption when exposed to lower relative humidities and sorption when exposed to higher humidities. In other words, at certain relative humidities the crackers are hygroscopic whereas at others they are hygro-emissive. It has been found useful to relate hygroscopicity and hygro-emissivity to typical ambient conditions. For this purpose a dividing line at 50% relative humidity has been chosen arbitrarily.

With this concept in mind, a hygroscopic product is defined as a product that has an equilibrium relative humidity (ERH) of less than 50% and a hygro-emissive product as one that has an equilibrium relative humidity of over 50%. Obviously, therefore, one index

[a] Presented before the Eleventh Annual Meeting of the IFT, New York, N. Y., June 19, 1951.

of the hygroscopic or hygro-emissive nature of a product lies in the value of the ERH.

It is possible for two products of different moisture contents, as manufactured, to have the same ERH at the same temperature and yet be quite different in their tendency to attract or give off water vapor. Figure 2 shows this clearly. Here both products, A and B, have the same ERH at their initial points (that is, at the moisture contents at time of manufacture), but the overall general slopes of the curves for the two products between ERH's of 0 and 100% are quite different. Product A appears to have a greater capacity for absorbing moisture.

To some, the adjective "hygroscopic" implies the attraction of moisture at a very high rate. Therefore, rates of diffusion should also be considered in measuring hygroscopicity. Hence at least three factors must be considered in evaluating hygroscopicity. These are the value of the ERH, the capacity for sorption, based on the slope of the humidity-moisture equilibrium curve, and the rate at which moisture is sorbed by the product.

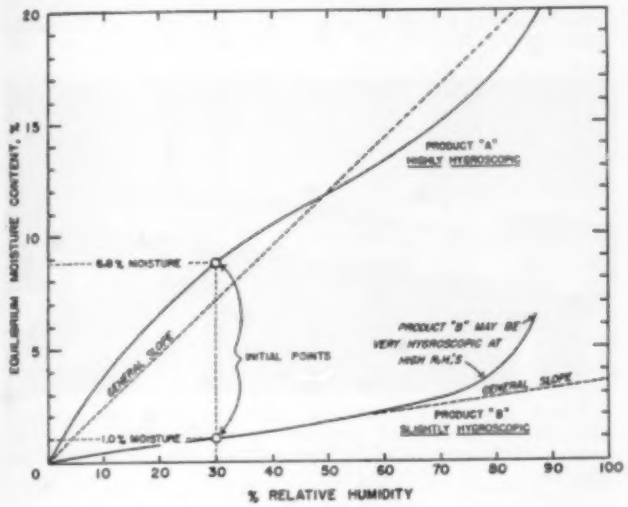


FIG. 2. Humidity-moisture equilibrium curves based on hypothetical products, as obtained by the graphical interpolation method. Note how two products having the same equilibrium relative humidity corresponding to their initial moisture contents are not necessarily equally hygroscopic.

Curves of the type shown in Figure 1 are of considerable value to the food packaging technologist because it is so easy to indicate on them how much moisture a product can gain or lose before it becomes unusable. An example of a curve giving this type of information is seen in Figure 3. The curve shown here was derived from data on powdered molasses, data obtained by the commonly used weight-equilibrium method of Wink *(8)*. This curve shows the initial point representing the moisture content and the ERH of the product as manufactured, the critical point where the product just begins to become unusable (in this case because of lumping), the safety range which is arbitrarily defined as the portion of the curve between the critical point and a point of 5% lower relative humidity, and the danger point which is the lower limit of the safety range. It is believed that if the packager aims at

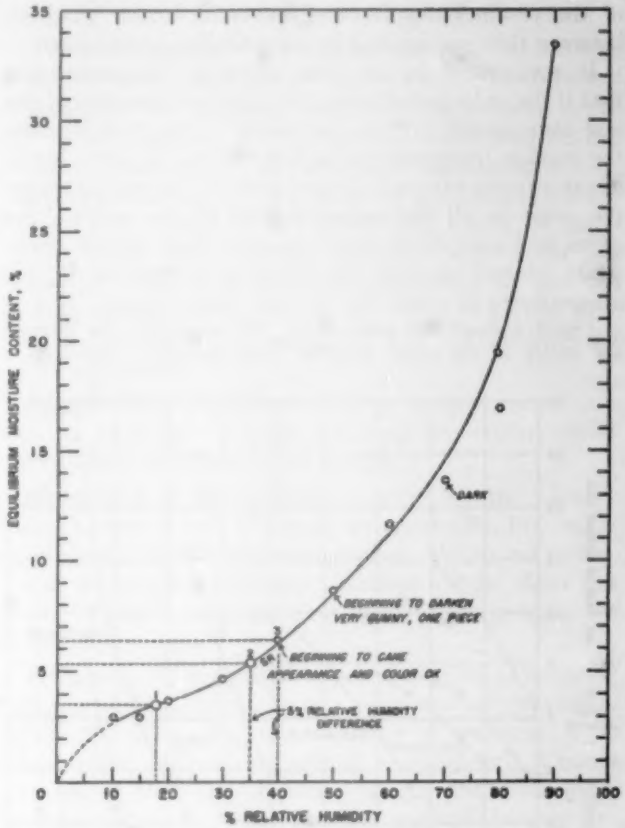


FIG. 3. Humidity-moisture equilibrium curve for powdered molasses at 25° C., as obtained by the Wink method using H_2SO_4 solutions. 1 = initial point (3.54% moisture — 17.9% R. H.); 2 = danger point (5.28% moisture — 35% R. H.); 3 = critical point (6.30% moisture — 40% R. H.); SR = safety range.

not permitting his product to reach this danger point, he will have a safe margin of error.

The critical point for lumping may not be the same as the critical point for other physical and chemical changes. It is obvious, however, that the lowest critical point will cause the most concern, and this point alone should be used in determining the danger point.

Some products, such as the gelatin dessert mix illustrated in Figure 1, pass through a wide span of ERH's before their moisture content increases by even 1 or 2%. Other products, such as the soda crackers and the dried chocolate sirup product (Figure 1), undergo a significant increase in moisture content along with the increase in ERH.

GRAPHICAL INTERPOLATION METHOD

It has been indicated how humidity equilibria data can be useful to the technologist. A method for obtaining data of this sort, called a graphical interpolation method, has been developed in the Food Technology Laboratories of the Massachusetts Institute of Technology to meet the need for a relatively simple, rapid test of this type *(4)*. This method is based on the principle that, at any given initial moisture content and under any given atmospheric relative humidity, the rate of gain or loss in the moisture content of a product increases with increasing difference between atmospheric and product relative humidities. This simply means that the rate of gain or loss in moisture content

of the product depends on the water vapor gradient between the product and its surrounding atmosphere.

It was found in any one series of measurements, that if the only variable was the relative humidity of the test atmosphere (if the durations of the test periods, the storage temperatures, and the initial weights, initial moisture contents, and surface areas of the samples were the same in all the measurements of the series), the gains or losses in moisture content were almost linear when plotted against the relative humidities of the atmospheres in which the samples were stored.

Figure 4 shows this graphically. The horizontal line through the center of the chart, labelled "zero baseline," represents a

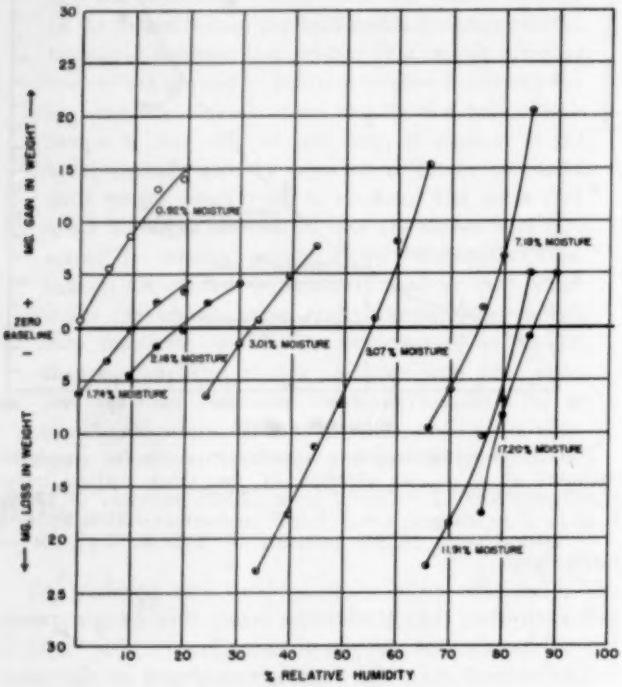


FIG. 4. Graphical interpolation isotherms for a dried chocolate sirup product, as obtained by the graphical interpolation method (1-hour test period at 25° C.).

condition where there are no gains or losses in weight of the product. The gains in weight of the product as a result of sorption of water vapor are shown as progressing upward and the losses in weight as a result of desorption as progressing downward from this baseline.

The point at which the relatively smooth curve intersects the baseline is obviously of considerable importance, for this is the point at which a true equilibrium exists between relative humidity and moisture content of product, with no gain or loss in weight. This point is the ERH or equilibrium relative humidity.

If the relative humidities were read at the points where the several curves intersect the zero baseline and if these values were plotted with reference to the initial moisture contents of the product represented by the curves, the result would be an equilibrium curve such as illustrated in Figure 1.

The following practical steps are necessary to carry out the determinations. First of all, it is essential to prepare eight or nine samples of any given product, each sample weighing about 60 g. These are to be conditioned to different moisture levels. Establishment of these moisture levels may be accomplished in an accelerated manner by placing some of the samples in a humidity oven at about 37.8° C. (100° F.) with a relative humidity of at least 90% and other samples in a vacuum oven at 40 to 50° C. (104 to 122° F.). After sufficient 60 g. samples of different moisture contents are obtained, they are temporarily stored in air-tight glass jars. Determinations of the moisture contents of 2-g. aliquots of these samples are then made in the vacuum oven.[b] The data in this report are based on moisture determinations at 70° C. (158° F.) and full vacuum (actually a 30-inch Hg reading) for a period of 16 hours, with three bubbles of dry air per second being pulled through the oven. These conditions were those specified by the Packaging Institute Food Committee for its recent round of tests.

For the measurements based on the graphical interpolation method, a series of special test units is necessary (Figure 5). Each unit consists of a glass crystallizing dish covered with a simple glass plate. A layer of heavy stopcock grease provides the closure between dish and cover. The samples of the food product to be tested are placed in metal pans approximately 3 inches in diameter, which are in turn placed on glass support platforms in the middle of the test units.

The proper relative humidity for the atmosphere in each test unit is supplied by placing in the bottom of each unit a solution of sulfuric acid of appropriate strength. The strengths of the solutions to provide the desired relative humidities at 25° C. (77° F.) are based on a table of specific gravities of sulfuric acid *(4)*. A rigid technique has been developed to insure accurate specific gravity readings for the mixtures. Precision hydrometers are used, and the necessary temperature corrections are made. To provide 0% relative humidity, Dehydrite is suggested as a desiccant. Phosphorous pentoxide was originally used, but with less success.

In an actual test, the humidity control units are arranged in a row from left to right (Figure 5), in the order of increasing

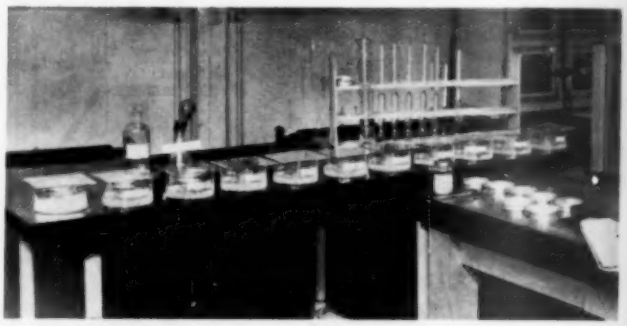


FIG. 5. Suggested arrangement of equipment for the graphical interpolation method of determining equilibrium relative humidity. Hydrometers in background are used in making acid solutions.

relative humidities of the test atmospheres. Five-gram portions of each 60-g. product sample are carefully but rapidly weighed on a Chainomatic balance and quickly placed in the appropriate test units. As soon as one test pan is in place, the next one is prepared. This operation requires from 2 to 4 minutes per unit.

Ordinarily each sample is removed from its test unit and weighed again exactly one hour after it is placed in the unit. From the data obtained in this way for the several 5-g. samples, the gains or losses in weight are plotted against relative humidity for the sample of the particular initial moisture level being studied, and a graphical interpolation curve (isotherm) is drawn, as illustrated in Figure 4.

This same procedure is repeated for the same product at several other initial moisture levels, and in each instance the ERH is interpolated at that point where the smooth curve intersects the zero baseline. These ERH's are then plotted with reference to the initial moisture contents of the samples to give a humidity-moisture equilibrium curve such as illustrated in Figure 1.

Experimental results indicate an acceptable consistency in the individual points constituting the graphical interpolation

[b] In a previous report on the graphical interpolation method *(4)*, the authors suggested measuring moisture contents of products in a vacuum oven at 70° C. and 30-inch Hg for 6 hours. All data reported were based on these conditions.

isotherms. In general, any fluctuations in these points from the smooth curve will be distributed randomly on either side of the isotherm, and an occasional widely fluctuating value will have no appreciable effect on the general trend of the curve.

Under one particular set of conditions with a gelatin dessert powder it was found that the coefficient of variation (V) of typical weight changes was 8.6% when 10 replicates were all exposed to the same relative humidity in different humidity control units. The coefficient of variation is simply the standard deviation (σ) expressed as a percentage of the mean. With these same ten samples, the maximum and minimum gains in weight were about 15% above and below the mean.

PRECAUTIONS TO BE OBSERVED

Temperature changes have some effect on the ERH. Experiments on a small scale indicated that for some typical powdered products a rise in temperature of 1° C. caused a rise of approximately 0.5% relative humidity. This holds true, however, only in the range of temperatures close to 25° C. (77° F.).

When powdered products are used, slight variations in the weights of samples are more significant than slight variations in the surface area of the tamped-down product. In an experiment with a gelatin dessert powder, it was found that the weight of sample was the important factor affecting the diffusion rate until sample weights of about 40 g. were used, at which time surface area became a limiting factor and weight of sample had little or no influence.

It might be stated, then, that for small amounts of powdered product such as the 5 g. suggested in this procedure, consistency in the weight of the sample is more important than consistency in the surface area, at least in the 3-inch diameter pans used in the graphical interpolation method. With increasing amount of sample, the importance of the surface area of the sample relative to its weight becomes greater until at a certain point the diffusion rate becomes independent of weight and dependent largely upon surface area. This observation holds only for one-hour test periods, however.

According to this graphical method, moisture contents of samples cannot be calculated from the amounts of moisture gained or lost at equilibrium, as they can be in the weight-equilibrium method. The moisture contents of all samples must be determined by accurate analyses.

Once a pan has been removed from one of a series of control units for final weighing, all other pans of the series must be weighed in turn after test periods of exactly the same duration. This may occasionally lead to some inconvenience and the spoiling of a run, if the technician is interrupted.

Many products require slightly specialized treatment, because of their peculiar shapes, forms, or physical states. For example, ordinary liquids are handled on a volume basis rather than on a weight basis. Products such as marshmallow have to be filled brimfull into special cups, as they cannot otherwise be readily handled on a volume or weight basis.

Because the method is based on interpolation of data, it is possible to make a slight error in reading the ERH from a graphical interpolation isotherm when insufficient points are plotted.

ADVANTAGES OF METHOD

The advantages of the graphical interpolation method, which are many, may be partially enumerated as follows:

Rapidity. A complete humidity equilibrium curve can be obtained in two days or less, depending on the number of technicians and analytical balances available. The determination of each point on the humidity equilibrium curve requires only about 1-¼ hours. A satisfactory curve can frequently be drawn with about five points, in which case sufficient data could be obtained in 6-¼ hours. This time is in addition to the time needed for conditioning samples and for determining moisture contents.

Simplicity of Procedure. Highly trained technicians are not essential. Anyone knowing the rudiments of curve-fitting should have no trouble.

Simplicity of Equipment. Except for the Chainomatic balance which is almost indispensable, the equipment used is simple and inexpensive. When not in use, it can serve other purposes. Because of the short test period of one hour, a constant-temperature room is not absolutely essential.

Accuracy. The method appears to give as accurate and reproducible results as any other method previously described. Naturally the accuracy is greatest when temperature fluctuations are at a minimum. Certain phenomena that may seriously affect results in equilibrium tests over long storage periods become insignificant in this short method. These include the danger of sulfuric acid acting to neutralize volatile bases or to oxidize the product *(3)*. Adsorption of molecular sulfuric acid may also take place to some extent over long test periods. In the study of some products, these effects may be of significant proportions.

Versatility. This method is versatile. For example, samples may be exposed for periods longer than one hour, if convenient, just so long as all samples of the same initial moisture content are under test for the same period of time.

Case-Hardening Eliminated. By case-hardening or skin-sealing is meant the phenomenon where the moisture content of the exposed surface of the product is different from that of the unexposed portion. When the outside portion becomes caked, this may act as a barrier for moisture sorption or desorption of the entire mass of the product. With reasonable care in making sure of the homogeneity of samples prepared for this new method, there is little possibility, even in the case of products of high moisture content, that skin-sealing or case-hardening will become a problem in such a short test period as one hour.

Unlimited Selection of Test Relative Humidities. The use of sulfuric acid solutions enables the technologist to select any relative humidity in which he is interested for his test solution, provided it is below 90%. It is difficult to establish consistently accurate relative humidities above 90%. Salt solutions do not permit much flexibility, especially in the range of low relative humidities. Furthermore, there is considerable disagreement in the literature with regard to the ERH's of many supersaturated salt solutions *(1, 6, 9)*.

Avoidance of Mold Growth and Enzymatic Breakdown. Frequently in weight equilibrium techniques requiring prolonged exposure periods, the problem of mold growth at high humidities becomes serious *(7)*. With this rapid method it is possible to carry out studies on such items as fresh and processed meats and products in which enzymatic breakdown might be serious. Obviously no weight equilibrium method could be used with meats, because of enzymatic and bacterial actions in meat kept over long periods of time.

Moisture Determination for Quality Control. Where a standard humidity-moisture equilibrium curve for a given product has been prepared with a large number of points, this curve can be used as a means of rapid determination of the moisture content (to one decimal place) of any sample of the same product at any given relative humidity. The standard curve should be based on a suitable reference method for moisture determinations, such as the vacuum oven method or the Karl Fischer titration technique. Makower and Myers *(5)* have pointed out the desirability of using vapor pressure data as substitutes for moisture data in dehydrated vegetables. ERH data are simply vapor pressure data expressed in a different way.

ERH of Product as Packaged. In many cases the technologist will be interested merely in knowing the ERH of a product at the moisture level at which it is produced and packaged. The older weight equilibrium method requires the completion of at least a substantial portion of the humidity equilibrium curve. This means that numerous weighings must be made until weight equilibrium is reached. From this partial curve the ERH for the product of the moisture content in question must then be read. With this graphical method, acurate results can be obtained by making a few weighings at relative humidity increments of approximately 5%. For example, if it were expected that the probable ERH of a product was between 10 and 30%, samples would be exposed to relative humidities of only 10, 15, 20, 25, and 30% and the ERH could be determined rapidly by graphical interpolation, or perhaps by extrapolation if the plotted points should fall outside the expected range of relative humidities.

Effective Evaluation of Formula Changes. In the past when minor changes were made in the formula of a product, it was often necessary to wait a month or two until data on the humidity equilibria could be obtained, before an evaluation could be made of the effect of these formula changes on the hygroscopicity of the product. This has often been a discouraging and expensive delay. With this new method, the effect of changes in formula on product hygroscopicity can be studied at the optimum time for effective evaluation, that is, within a few days after the changes have been made.

Applicability to Wide Variety of Products. This method is applicable to any type of material, whether powders, solids, liquids, or heavy sirups. The authors have tried it successfully with a powdered potato product, pure soluble coffee, a dried chocolate sirup product, soda crackers, and flavored gelatin dessert mixes. Full humidity equilibrium curves have been prepared for these products. In addition, partial studies have been made on fudge, powdered molasses, candy wafers, confectioner's corn sirup, large whole prunes, marshmallow, ice cream mix, dried whole egg, jelly eggs, Dehydrite, and even water.

The method is especially suitable for unit products such as pieces of candy, crackers, and the like, which are reasonably constant in both weight and surface area. With unit products it is possible to use the entire unit in the test, rather than a pulverized form. Slight variations in the weights of the unit products are relatively insignificant. Testing such products as units is much more realistic from a packaging point of view than testing them in a pulverized form.

The method should also be applicable to such nonfood items as tobacco, paper, plastics, fertilizers, and pharmaceuticals.

SUMMARY

To design the best functional package for protection of foods and food products during storage, a study is necessary of the humidity-moisture equilibria of such products. To ascertain the humidity-moisture equilibria of a product, the equilibrium relative humidity (the storage relative humidity at which a product will neither gain nor lose moisture) must be determined for that product at each of several different moisture contents.

A graphical interpolation method for measuring equilibrium relative humidity is described, which is simple, inexpensive, and rapid. This method is based on the observation that if the only variable in any one series of measurements is the relative humidity of the test atmospheres in which the samples of a given product are stored, the gains and losses in weight of the product samples under these test conditions will be almost linear when plotted against the relative humidities. This means that the duration of the test period, the storage temperature, and the initial weight, initial moisture content, and surface area of the sample must be the same in all measurements of the series. The gains and losses in weight noted under such conditions are plotted graphically with reference to the relative humidities, and a curve is drawn through the plotted points. The equilibrium relative humidity is then read at the point where the curve intersects a "zero baseline" representing no gain or loss in weight.

With this method accurate data for determining the equilibrium relative humidity of a product at a given initial moisture content can be obtained in approximately one hour.

Repetition of this series of measurements for the same product at several other initial moisture levels provides data for drawing a humidity-moisture equilibrium curve. About two days are required to obtain such a curve, depending on the number of points desired for the curve.

The method is applicable to a wide variety of foods such as powders, sirups, liquids, and solids (including meats and bread). It is especially suitable for unit items such as crackers and pieces of candy that are reasonably constant in both weight and surface area.

Acknowledgments

The authors wish to express their sincere appreciation to Prof. Emeritus Warren K. Lewis of the Department of Chemical Engineering, to Prof. George de Santillana and Prof.

Theodore Wood, Jr., both of the Department of English and History, and to Prof. William C. Bauer and Mr. Felix Bronner, both of the Department of Food Technology, Massachusetts Institute of Technology, for their helpful suggestions with regard to the terminology used in this paper.

LITERATURE CITED

1. Carr, D. S., and Harris, B. L. Solutions for maintaining constant relative humidity. *Ind. Eng. Chem.*, **41**, 2014 (1949).
2. Elder, L. W. Functional package design. *Chem. Eng. News*, **27**, 2682 (1949).
3. Hubbard, M. R. Evaluation of modern apparatus and methods for moisture determination in dehydrated foods. S.B. Thesis, Mass. Inst. Tech., 10 (1943).
4. Landrock, A. H., and Proctor, B. E. Measuring humidity equilibria. *Modern Packaging*, **24** (6), 123 (1951).
5. Makower, B., and Myers, S. A new method for the determination of moisture in dehydrated vegetables. *Proc. Inst. Food Technol.*, 156 (1943).
6. Stokes, R. H., and Robinson, R. A. Standard solutions for humidity control at 25° C. *Ind. Eng. Chem.*, **41**, 2013 (1949).
7. Ulrich, J. A., and Halvorson, H. O. The possibilities of the growth of *Clostridium botulinum* in canned bread. *Annual Report Hormel Inst., Univ. Minn.*, 45 (1948-49). Univ. Minn. Press, Minneapolis.
8. Wink, W. A. Moisture equilibrium. *Modern Packaging*, **20** (6), 135 (1947).
9. Wink, W. A., and Sears, G. R. Instrumentation studies. LVII. Equilibrium relative humidities above saturated salt solutions at various temperatures. *Tappi*, **33** (9), 96A (1950).

Retarding the Loss of Red Color in Cayenne Pepper With Oil Antioxidants[a,b]

L. O. VAN BLARICOM and J. A. MARTIN
South Carolina Experiment Station, Clemson, South Carolina

(Manuscript received March 13, 1951)

The loss of red color in dried and ground cayenne pepper was controlled in various degrees by the addition of antioxidants which are used in fats and oils to prevent rancidity. The following materials were effective in various degrees in controlling the discoloration of cayenne pepper: lecithin; a preparation containing butylated hydroxyanisole, hydroquinone, and citric acid; a preparation containing butylated hydroxyanisole, propyl gallate, and citric acid in propylene glycol; a preparation containing corn oil, lecithin, and propyl gallate; and sesame seed ground with the pepper. Those antioxidant preparations available as a dry powder or solutions which were not oil-soluble gave little or no control.

South Carolina produces approximately 1,000,000 pounds of cayenne pepper *(Capsicum frutescens)* annually. After harvesting, the pepper is usually dried in tobacco barns at 54 to 66° C. (130 to 150° F.) and then shipped to manufacturers. After grinding, the dried pods of the pepper lose their normal red color, through a period ranging from a few weeks up to 5 or more years, turning various shades of undesirable brown. For this reason red color retention is of considerable economic importance to the pepper growers and processors in South Carolina.

In 1942 a breeding program was initiated at Clemson Agricutural College to develop desirable strains and varieties of cayenne pepper for the industry. The loss of the red color of pepper was observed by Martin *(5)* shortly after the breeding program was undertaken. This loss of red color or discoloration varied between individual plants of the same strain and between different strains. Over a period of 8 years it has been observed that certain individual plants possessed remarkable ability to retain the red color in the dried pods. In fact, a few samples which were prepared in 1944 still retain their bright red color, but a majority have lost all of their red color.

The factors responsible for pungency are not correlated with color retention since the pungent component, capsaicin, is retained for long periods and is still present in samples which have lost their original color.

There is very little information on the chemical composition of the red pigments in cayenne pepper. However, Mayer and Cook *(7)* cite the work of Zechmeister, Cholnoky, and Karrer who found 6 poylene alcohols, 6 organic acids, and 2 polyene hydrocarbons present in the pigments of Spanish pepper. They state that one of the constituents is capsanthin, and the separated capsanthin is slowly oxidized in air. Brown *(1)* also found capsanthin in Perfection pimiento.

In 1942 a series of experiments were conducted by Van Blaricom and Martin *(8)* in which the peppers were treated in various ways before drying at a temperature of 66° C. (150° F.). These treatments included blanching in steam, exposing to the fumes of sulphur dioxide, dipping in solutions of sodium sulphite, dipping in solutions of citric acid, and combinations of these various chemicals and treatments. With the exception of sulphur dioxide or sodium sulphite the various treatments had no significant results. The sulphur dioxide radical appeared to hasten the loss of the red color.

In 1947 several experiments were conducted by Martin *(6)* in which four varieties of peppers, Louisiana sports and three cayenne strains, were grown in South Carolina at Clemson, Charleston, Florence, Blackville, and in North Carolina at Highlands. These experi-

[a] Technical contribution No. 190, South Carolina Agricultural Experiment Station, Clemson.

[b] This report is being made for its scientific interest. Anyone contemplating the use of the materials described should consult the federal, state, and local food regulatory agencies governing the use of such materials in food products.

ments showed wide variations in the rate of discoloration. In general, the results showed that ground samples of the peppers grown on heavy soils in the Piedmont area possessed superior color and retained the red color longer than the peppers grown on light soils of the coastal areas. Since these peppers were grown on different soil types and under different environmental conditions, it appeared that the deficiency of certain physiological factors might be responsible for some of these differences in color retention and variation. However, results of other tests involving minor element studies with pepper at Clemson and Florence show wide differences in rate of discoloration regardless of the variety, the location where grown, or the kind of fertilizer used.

Peppers are high in ascorbic acid, ranging from 79 to 324.7 mg. per 100 g. on 26 lots of fresh samples of peppers at Clemson. During drying most of this ascorbic acid is lost, and there seems to be no correlation between original ascorbic acid content and color retention.

Peppers are relatively high in oil, and this fact appears to be correlated with color retention and discoloration. In 1944, seven strains of dried Cayenne pepper which had been ground with the seed were selected because the samples had lost their color, and seven similar strains were selected because they had retained most of their red color. Fat and oil determinations were then made on these fourteen samples. The seven samples which retained most of the red color ranged from 10.05 to 13.79% in fat and oil, while the seven samples which had lost their color ranged from 27.96 to 30.75%. This relationship is further confirmed by the fact that most of the oil in the ground pepper comes from the seed; and when the pepper is ground without the seeds, the color seems to be much more stable and to require a much longer time to fade out.

Many observations during these investigations have shown that light hastens discoloration. Samples stored in the dark retain their red color much longer than those stored in the light. Samples in glass jars frequently lose their color more quickly on the side facing light from an outside window.

Pods which are injured, broken, or cut before drying discolor more rapidly than uninjured pods. The loss of color frequently begins at a broken edge and spreads gradually to the rest of the pod. Ground pepper in glass sample jars usually begins to discolor at several points and then spreads to the surrounding area. This often gives a spotted appearance to the sample. Occasionally sample jars of ground pepper prepared in an identical manner will show variation in the amount of discoloration, particularly in the time the samples begin to discolor. After discoloration begins, the rate of discoloration seems about the same for all lots of identical samples.

EXPERIMENTAL PROCEDURE

The above experiments and observations indicated that the loss of the red color of pepper was probably the result of oxidation and that it might also be connected in some way with the stability of the oil of the peppers. For this reason a series of experiments were begun in the fall of 1948 in which ground cayenne pepper was treated with oil antioxidants which are used to prevent rancidity and oxidation in fats and oils *(9)*. The antioxidant materials used were: (a) nordihydroguaiaretic acid (N.D.G.A.); (b) an oat flour product; (c) lecithin; (d) a preparation containing butylated hydroxyanisole, hydroquinone, and citric acid (Tenox 1); (e) a water soluble preparation containing propylene glycol, propyl gallate, and citric acid (Tenox BHA); and (f) a preparation containing corn oil, lecithin, and propyl gallate.

Because of the difficulty in mixing it with the pepper the oat flour concentrate was used at only one concentration. Lecithin, in the form which was available, also mixed with difficulty.

In 1948 the antioxidants were used at three different concentrations. Samples of a single strain of ground pepper weighing 300 to 400 g. were used for each test. The antioxidant was added at the concentrations shown in Table 1 and mixed thoroughly by hand with a mortar and pestle. The pepper was placed in small jars, sealed, and placed in a room with diffused outside light for observation. Ratings of the samples were made at the end of one year by visual observation on the basis of the percent of color retained. The standard for the ratings was a freshly ground sample of the same strain of pepper from the following year's crop.

Information obtained after the 1948 experiments were begun suggested further testing in the fall of 1949. Consequently, in addition to some of the antioxidants used in 1948, the following were used: (a) sesame oil, refined and bleached; (b) sesame oil, refined, bleanched, and deodorized; (c) sesamol *(2)*; (d) sesame seed; (e) butylated hydroxyanisole, propyl gallate, and citric acid in propylene glycol; and (f) mineral oil alone and in combination with some of the other antioxidants. The sesame seed was ground with the dried pepper. The other materials were used as described in the 1948 experiments. Mineral oil was added to determine whether it would serve as an oil vehicle for those antioxidants which were not effective in the 1948 experiments.

Five check samples were used in 1948-1949, and six samples were used the following year. As shown in Table 1, all except one check sample rated 20% at the end of one year, while one sample was rated 70% from the 1948-1949 lot. Thus one control sample, which had been prepared from the same strain of ground pepper, mixed thoroughly and placed in the jar in as nearly an identical manner as possible, was completely different from the other controls. This erratic and unpredictable behavior has been noticed repeatedly during the eight years that the authors have been observing pepper colors. This behavior suggests that the discoloration may be one of autoxidation or a catalytic reaction brought about by the decomposition products.

DISCUSSION OF RESULTS

As shown in Table 1, some of the antioxidants were quite effective in retaining the red color in the pepper, while some of the materials had little or no effect. The oat flour product when used alone showed little evidence of retaining the color at any concentration in either year. All four samples rated 20%, the same as all the control samples.

In 1949 the sample containing 5% mineral oil with the oat flour rated 40% as compared to 20% in the control. This difference is not great, but it suggests that oat flour might be effective if a proper vehicle were supplied to make intimate contact between the color pigments and the antioxidant. The oat flour concentrate was so thick and viscous that mixing was extremely difficult. However, with a rating of 40% it shows some control.

Lecithin was also quite effective. However, this product is also quite viscous in the form which was used and mixed with difficulty.

Propyl gallate in the water soluble preparation rated only 30% at the highest concentration used. In con-

TABLE 1

Color ratings of cayenne pepper treated with antioxidants

Antioxidant material	Trade name	Percent of antioxidant or compound used	Percent of red color retained after 12 months 1948-49	1949-50
Oat flour product [c]	Avenex 7	0.5	20	....
		2.0	20	....
		8.0	20	....
		5.0	....	20
Oat flour product plus 5% mineral oil		5.0	....	40
Oat flour concentrate [c]	Avenex concentrate	0.5	40	....
Lecithin [d]	Alcolec	0.25	70	....
		1.0	60	....
		4.0	70	....
Propylene glycol, propyl gallate and citric acid [e]	Griffith's Water Soluble G-4 Concentrate	0.02	20	....
		0.1	20	....
		0.4	30	....
Lecithin, propyl gallate, and corn oil [e]	Griffith's G-4 Type C Concentrate	0.25	30	....
		1.0	30	....
		4.0	80	....
		0.5	....	20
Nordihydroguaiaretic acid, 1% solution [f]	N. D. G. A.	0.1	20	30
		0.4	20	....
		1.6	40	....
Nordihydroguaiaretic acid, dry powder	N. D. G. A.	0.01	20	....
Nordihydroguaiaretic acid, 1% solution plus 4% mineral oil	N. D. G. A.	0.1	....	50
Butylated hydroxyanisole, hydroquinone [g] and citric acid	Tenox 1	0.05	40	20
		0.2	60	30
		0.8	90	40
Butylated hydroxyanisole, propyl gallate citric acid, and propylene glycol [e]	Tenox BHA	0.05	....	30
		0.2	....	40
		0.8	....	70
Mineral oil		0.25	....	20
		1.0	....	20
		4.0	....	30
Sesame oil, refined and bleached [h]		0.25	....	20
		1.0	....	20
		4.0	....	30
Sesame oil, refined, bleached, and deodorized [h]		0.25	....	20
		1.0	....	30
		4.0	....	40
Sesamol, 1% in ethyl alcohol [h]		0.2	....	40
Sesame seed ground with pepper		2.0	....	40
		5.0	....	60
		10.0	....	80
Untreated checks		....	20.0	....
4 samples		....	70.0	....
1 sample		....	....	20
6 samples				

[c] Supplied by The Quaker Oats Company, New York.
[d] Supplied by American Lecithin Co. Inc., Long Island City, New York.
[e] Supplied by The Griffith Laboratories, Chicago, Illinois.
[f] Supplied by Wm. J. Stange Co., Chicago, Illinois.
[g] Supplied by Tennessee Eastman Corporation, Kingsport, Tennessee.
[h] Supplied by Southern Regional Research Laboratory, New Orleans, La.

trast to this, the propyl gallate with lecithin in corn oil rated 80% at the highest concentration.

Nordihydroguaiaretic acid (N.D.G.A.) showed very little control except in one lot which rated 40% when 1.6% of a 1% alcohol solution was used. However, when 4% mineral oil was added, an increase from 30 to 50% was noted.

Mineral oil by itself showed little evidence of controlling discoloration. However, when added with oat flour and N.D.G.A., which were not effective as a dry powder, the mineral oil apparently provided a vehicle which made these two antioxidants more effective.

Tenox 1 and Tenox BHA were both effective in retarding discoloration. Adding 0.8% of these two antioxidants resulted in 90 and 40% retention for Tenox 1, and 70% retention for Tenox BHA.

Two samples of sesame oil when used up to 4% were not very effective. Concentrations above this were not tried; but at 4% some oiliness and stickiness were observed in the samples, and it is doubtful that concentrations above this figure could be used.

Only a small amount of sesamol was available in a 1% alcohol solution. This solution used at the level of 0.2% gave 40% color retention at the end of a year. In the proper oil vehicle and higher concentrations employed, this antioxidant would probably be quite effective.

Sesame seed ground with the pepper gave quite effective control. When 10% sesame seed was added, 80% retention was obtained. This seed was easy to grind with the pepper. Apparently, the oil in the sesame seed which contains the antioxidant, sesamol, was effective in controlling the loss of red color in the ground pepper. The addition of sesame seed to the pepper during grinding appears to be a simple, effective, and economical method of preventing the discoloration of pepper.

SUMMARY

The following materials were found effective in various degrees in controlling the discoloration of cayenne pepper: Lecithin; a preparation containing butylated hydroxyanisole, hydroquinone, and citric acid (Tenox 1); a preparation containing butylated hydroxyanisole, propyl gallate, and citric acid in propylene glycol; a preparation containing corn oil, lecithin, and propyl gallate; and sesame seed ground with the pepper.

Other antioxidants and compounds, available as powder or flours would probably be effective if the proper oil vehicle were used.

LITERATURE CITED

1. Brown, W. L. A contribution to the chemistry of pepper pigments. *J. Biol. Chem.*, 110, 91 (1935).
2. Budowski, P. J. Sesame Oil III. Antioxidant properties of sesamol. *J. Am. Oil Chemists' Soc.*, 27, 264 (1950).
3. Dugan, L. R., Jr., Kraybill, H. R., Ireland, L., and Vibrans, F. C. Butylated Hydroxyanisole as an antioxidant for foods made with fat. *Food Technol.* No. 1, 4, 457 (1950).
4. Kraybill, H. R., Beadle, B. W., Vibrans, F. C., Swarty, Venona, Wilder, O. H. M., and Rezabek, Helen. Am. Meat Institute Foundation, *Bull.* 2 (1948).
5. Martin, J. A. Pepper investigations—Cayenne pepper improvement. South Carolina Exp. Sta., *Annual Report*, 56, 45 (1944).
6. Martin, J. A. Unpublished data. South Carolina Exp. Sta., (1947).
7. Mayer, Fritz. *The Chemistry of Natural Coloring Matters.* Translated and revised by A. H. Cook (1943), Reinhold Publishing Corporation, New York.
8. Van Blaricom, L. O., and Martin, J. A. Effect of predrying treatments and varying storage conditions upon the retention of color by Cayenne pepper. S. C. Exp. Sta., *Annual Report*, 57, 39 (1945).
9. Vibrans, F. C. Lard takes on new properties. *Food Inds.*, 20, 855 (1948).

Food Preservation with Antibiotics: The Problem of Proof

E. J. CAMERON AND C. W. BOHRER [a]
National Canners Association, Washington, D. C.

(Manuscript received May 5, 1951)

A discussion is given of the elements of proof that will be necessary to establish the acceptability of an antibiotic in food preservation. First, there must be laboratory proof of the ability of the antibiotic to destroy spores of Clostridium botulinum and saprophytic spoilage organisms, confirmed by pilot scale experimentally inoculated packs. When this is accomplished consideration will extend concurrently into the industrial and public health fields. The industrial phase will include studies on stability and canning technology. The public health and regulatory phase will survey the public health implications of continuous use of the antibiotic and will consider principally its toxicology.

It seems to be generally agreed that there is no early prospect of using antibiotics for the preservation of canned foods which are presently sterilized by high temperature processing. During the past year (1950) several investigations conducted by various groups were directed to studies of the sterilizing effects of a number of antibiotics, including subtilin against spore-forming organisms that must be controlled by the canning process. None of them show promise.

Among the more recent reports on studies relating to preservation by antibiotics are those by Bohrer *(1)*, Burroughs and Wheaton *(2)*, Olcott *(4)*, and Cameron *(3)* which together provide a comprehensive story of the recent studies and results upon which is based the evaluation of antibiotics as food preserving agents. Collectively, these papers also provide a substantially complete bibliography.

Although there is not now any antibiotic which shows promise as a substitute for high temperature processing, there is indisputable evidence that certain antibiotics do affect spore germination in some manner. It is evident from the data that the effect is at least partially bacteriostatic or sporestatic, and that this inhibitive condition may be transient. For example, in a medium the antibiotic may restrain growth of test spores for a period of several days followed by quick growth which may occur without appreciable reduction in the potency of the antibiotic. In such a case, the action of the antibiotic would be considered to be bacteriostatic. Certainly it has not been lethal to all spores against which it has acted.

To illustrate further from the results of an actual test, (Table 1) we may inoculate a medium with spores of "flat sour" organism No. 1518. The inoculated medium shows a count of 600,000 spores per ml. Subtilin is added to the level of 50 p.p.m. and the medium is then heated mildly [to about 93° C. (200° F.)]. After a short holding period we find that only 100,000 spores may be counted by the plate counting technique. What has happened to the 500,000 spores? Are they dead or only inhibited from growth?

Now if we hold the inoculated medium for 3 hours and make another count there is a further reduction in the number of countable spores to 40,000. But when we heat the tube, then count again, there is a recovery almost to the level of the 100,000 spores found after the first subtilin-heat treatment.

TABLE 1

Effect on flat sour organism No. 1518 of alternate heat shocks and incubation with subtilin or gentian violet

Incubation, hours	Number of organisms detectable with			
	subtilin, 50 p.p.m.		gentian violet, 50 p.p.m.	
	Before heating	After heating [b]	Before heating	After heating [b]
0	600,000	100,000	30,000	9,000
3	40,000	90,000	2,500	10,000
6	25,000	90,000	500	8,500
24	25,000	80,000	.....	.....
48	25,000	90,000	.....	.....
192	20,000	30,000	.....	.....

[b] Tubes held in boiling water for 5 minutes.

From this we know that at least 60,000 spores were merely inhibited. The heat shock apparently did something to break the stasis. But the inhibitive principle re-established itself on additional holding and then broke again on heating, as illustrated by the interval counts up to 48 hours.

But there is an unresolved question with respect to the fate of the spores represented by the original reduction in count of 500,000 spores. We cannot conclude whether they are actually destroyed or merely restrained from growth and it is important to the problem to determine, if possible, just how the antibiotic affects the spores that do not develop.

In formulating an hypothesis to explain the subtilin effect, it is interesting to compare the action of subtilin on the test organism with that of Gentian violet, a well known bacteriostatic agent. As shown in Table 1, the effects of the two agents seem remarkably alike. If we assume that Gentian violet is non-lethal, it is reasonable to conclude also that the action of the antibiotic is largely, if not entirely, bacteriostatic.

In testing the effect of subtilin on *Bacillus thermoacidurans*, which is the organism responsible for "flat sour" spoilage in tomato juice, we have encountered what may be an actual killing effect on the spores.

Observations such as these demonstrate that the antibiotic exerts some positive action against spores of important spoilage organisms. Consequently we believe that studies in this field should not be suspended at this point merely because no ideal antibiotic for preservation

[a] Presented at the Eleventh Annual Meeting of the IFT at New York, N. Y., June 20, 1951.

has yet been discovered. In our industry group there is general agreement that the advantage that would come from the ability to substitute low temperature for high-temperature treatment of certain products amply justifies the continued attention that is planned for this problem. It is our understanding that other agencies, and particularly the Bureau of Agricultural and Industrial Chemistry, are planning to continue and enlarge studies in this area with the common hope that the "ideal" antibiotic may be found.

Quite early in our 1950 investigations a decision was reached to join the laboratories of the National Canners Association with those of the American and Continental Can Companies in an industry project on the problem. This decision follows logically from the pattern of investigation which has been applied to thermal processing studies. As has been done in the case of thermal processing projects, a schedule of work was outlined which was designed to give priority to the study of essential requirements.

This industry group has concluded that in a continued study of the preservative effects of antibiotics a decision must be reached as to what should constitute proof of the efficiency of an antibiotic as a preservative. The control plan which was adopted as a guide in the antibiotic investigations calls for studies in four principal areas of interest:

1. botulism studies (types A and B) including laboratory tests, and inoculated packs;
2. spoilage studies (non-pathogenic spore-forming types) including laboratory tests, and inoculated packs;
3. the industry phase including chemical studies, and technological studies; and
4. the regulatory phase consisting of the public health aspects, and the legal aspects.

First, it is logical that initial attention be given to laboratory testing to determine certain basic bacteriological facts. Under laboratory conditions it has to be shown that the antibiotic is capable of destroying spores of organisms that must be controlled in conventionally heat processed canned foods. It must be demonstrated beyond doubt that spores of *Clostridium botulinum* will be destroyed. If the antibiotic does not pass this phase of testing there is no point in going further.

Second, assuming that laboratory tests with companion experimental packs demonstrate the ability of the antibiotic to destroy the spores of *Cl. botulinum* and the saprophytic spoilage organisms, it becomes necessary to extend consideration concurrently into studies in the industrial and public health fields. The industrial phase will include certain investigations such as studies of chemical composition and stability. These would logically be undertaken by the manufacturers of antibiotics. At the same time technological studies would have to be undertaken within the canning industry to establish canning techniques. These would include field trials, running through semi-commercial development and up to full commercial application.

The public health and regulatory phase of study would survey the public health implications of continuous use of the antibiotic and would consider principally its toxicology. Collaterally, the legal aspect would deal primarily with questions of compliance with food standards and regulations. Official approval of use in food products would require, initially, proof of efficiency against spoilage organisms and the meeting of public health requirements; after that the existing standards of identity for many products would have to be dealt with.

The work that is planned in the field of antibiotic preservation will cover the testing of a great number of these substances and in their study it would be illogical to attempt to enter all three areas of consideration at the same time. The requirements of proof and their order of essentiality are 1, the antibiotic must destroy spores of *Cl. botulinum;* 2, it must destroy spores of saprophytic organisms; 3, it must be non-toxic and otherwise meet public health requirements; 4, it must meet food law requirements, and 5, it must be economically practicable.

Returning to the mechanism of action against spores by antibiotics, we may have to decide whether "permanent inhibition" is the practical equivalent of spore destruction, especially in the case of *Cl. botulinum*. The difference cannot be determined absolutely where there is complete suspension of activity; as indicated in Table 1 by the initial spore reduction brought about by presence of subtilin and mild heat. No one can say whether this reduction represents death or inhibition.

On the other hand, in an experimental pea pack treated with subtilin and inoculated with flat sour spores, there was no spoilage during an incubation of 185 days, even though viability of the inoculum was established at will by culturing in media that did not contain subtilin. This presents a true picture of sustained inhibition. Whatever the attitude of technologists and public health authorities in regard to the equivalency of permanent inhibition and destruction in the case of the saprophytic spore formers, it is likely that, in the case of *Cl. botulinum*, there would be insistence on actual destruction, established by every known test for viability. Even in the case of partial acceptance of permanent inhibition as a principle of preservation, complete stability of the antibiotic would have to be established over a wide range of conditions of use in processing and storage.

Our control plan for investigating antibiotics indicates a liberal use of experimentally inoculated food packs to supplement laboratory techniques. As is the case in the reaction of spores to heat, there is a relationship between the number of spores exposed to the antibiotic and their sensitivity to it. This fact calls for some decision in reference to what constitutes a "reasonable" level of spore inoculum, especially in regard to *Cl. botulinum*. In the derivation of thermal processes very heavy inocula are employed.

While such a decision may be important in the future studies of preservation by antibiotics, it has not been of extraordinary importance in the subtilin investigations. Table 2 presents results from an experimental

TABLE 2
Botulinum spoilage in inoculated peas canned with subtilin—mild heat

Number of spores per can	Percent spoilage	
	Cl bot. No. 62-A	*Cl. bot.* No. 213-B
1-2 million	100	24
1000	100	0

pack of peas in which the product was inoculated at high and low levels with sports of *Cl. botulinum* types A and B. One to two million, and 1,000 spores per can (211 x 400) were used and the cans were heated to about 93° C. (200° F.). With the subtilin at a concentration of 20 p.p.m., 100% spoilage occurred at both levels of inoculation where *Cl. botulinum* Type A was the inoculum. In the case of Type B, spoilage was 24% at the high level and none at the low. These observations have incidental interest in demonstrating again the differences in sensitivity between strains within a species which have often been observed in other connections.

Where *Cl. botulinum* is concerned it is probable further that technologists and public health authorities would look for the destruction of unlimited numbers of spores. This criterion applies in the case of thermal processes for low acid products and we know of no good reason for a different standard for another kind of food preservation.

It is expected that in our future program for antibiotics the pattern of investigation which has been described will be utilized many times. Thus far it has been followed in testing several antibiotics, all of which have failed to meet the basic test, that is, the ability to destroy spores of *Cl. botulinum*. In the case of subtilin, to which most attention was directed, there has been further proof of ineffectiveness furnished by results of experimental packs which demonstrated that it lacked destructive ability not only against *Cl. botulinum* at a low level contamination, but also against saprophytic spoilage organisms.

LITERATURE CITED

1. Bohrer, C. W. N.C.A. experimental program with subtilin. Nat. Canners Assoc., *Information Letter*, 1325 (1951).
2. Burroughs, J. D., and Wheaton, E. Results of studies on the preservative action of antibiotics in processed foods. Nat. Canners Assoc., *Information Letter*, 1325 (1951).
3. Cameron, E. J. Use of antibiotics in preserving foods. To be published in *Proc. Am. Meat Inst. Found.* for 1951.
4. Olcott, H. S. Problems in the application of antibiotics to food processing. Nat. Canners Assoc., *Information Letter*, 1325 (1951).

Evaluation of the Thiobarbituric Acid Test as a Measure of Oxidized Flavor in Milk[a]

W. L. DUNKLEY
Division of Dairy Industry, University of California, Davis, California

(Manuscript received April 10, 1951)

Common tests for oxidative deterioration in fats and oils lack the sensitivity necessary for use as measures of oxidized flavor in milk and cream. The reliability of a method for the chemical estimation of oxidized flavor based on the formation of a red color when oxidized milk is acidified and heated with 2-thiobarbituric acid was investigated. The results demonstrate that the thiobarbituric acid (T.B.A.) test correlates closely with numerical flavor scores of milk samples having oxidized flavor of varied intensity.

Oxidized flavor is at present the most serious flavor defect of non-homogenized market milk in California. The extensive literature dealing with this defect has been reviewed by Brown and Thurston *(4)* and Greenbank *(5)*. Oxidized flavor is attributed to a mild oxidation of lipid materials in milk, particularly the phospholipids. Several other names have been used to describe the defect, among them "cappy," "cardboardy," "oily," "metallic," and "tallowy." In the dairy literature the term "rancid" is usually applied only to the defect resulting from hydrolysis of fat and related compounds and therefore does not include oxidized flavor.

Research on oxidized flavor in dairy products has been handicapped by the lack of a suitable chemical test for its estimation. Organoleptic evaluations must remain the final standard for flavor quality of milk, and they provide a satisfactory measure of oxidized flavor where other defects are not encountered. However, they are of limited value where several defects occur simultaneously. A chemical method for estimating intensity of oxidized flavor would be useful in such cases.

Lea *(9)* discussed the limitations of a number of chemical tests for the estimation of oxidative flavor deterioration in fats and oils. Tests such as the peroxide number determination, Kreis test, Schiff aldehyde test, Schibsted fat aldehyde value, and iodine number determination have found limited application in studies of dairy products such as butter and whole milk powder but are not sufficiently sensitive for use with milk and cream.

Results reported in a series of publications *(1, 2, 3, 7, 8, 12)* which described the application of a reaction involving thiobarbituric acid for the estimation of oxidation in various lipid materials indicated that the thiobarbituric acid test (referred to hereafter as the T.B.A. test) might be used as a measure of oxidized flavor in

[a] Supported in part by funds from the California Dairy Industry Advisory Board.

milk. The test is proving helpful in this laboratory in research on oxidized flavor and the mechanism of its development.

This paper describes three experiments designed to determine the reliability of the T.B.A. test as a chemical measure of oxidized flavor in milk. In experiments of this type, organoleptic measures of flavor intensity must be used as the ultimate standard even though the chemical test may be more dependable. For this reason, to aid in interpretation of the correlation between organoleptic scores and results of the T.B.A. test, the reproducibility and reliability of the scorers' flavor judgments were given critical consideration.

METHODS

The T.B.A. Test. The test is based on a reaction involving the formation of red color when oxidized milk is acidified and heated with 2-thiobarbituric acid. Ten ml. of the milk to be tested were pipetted into a 40 ml. centrifuge tube, 5 ml. of T.B.A. reagent (containing 0.02*M* 2-thiobarbituric acid and 2*M* phosphoric acid) added and mixed well. The sample was heated in a boiling water bath for 12 min., cooled rapidly in refrigerated water and extracted by shaking vigorously for 2 min. with 15 ml. of extraction mixture (2 parts isoamyl alcohol and 1 part pyridine) and centrifuging for 5 min. at a minimum of 3000 r.p.m. Part of the clear solvent layer was removed and its optical density at 535 mμ determined with a Beckman Model DU spectrophotometer. Results of the T.B.A. tests are reported as the optical density readings.

Copper causes some increase in optical density in the T.B.A. test. To provide a means of correcting the T.B.A. test for interference from copper, correction factors were determined by applying the test to a series of fresh milk samples containing varied amounts of added copper. In experiments in which oxidized flavor was induced by the addition of low concentrations of copper as copper sulfate, the correction factors given below were deducted from the optical density of the T.B.A. test: 0.1 p.p.m., 0.002; 0.2 p.p.m., 0.004; 0.5 p.p.m., 0.01; 1.0 p.p.m., 0.016; 2.0 p.p.m., 0.023; and 5.0 p.p.m., 0.034. In most cases the copper concentration did not exceed 0.5 p.p.m.

As a guide for interpretation of results reported below, in most cases the T.B.A. test of fresh milk was below 0.040, slight oxidized flavor was usually detectable when the T.B.A. test had increased to 0.050, and samples with a distinct oxidized flavor usually had T.B.A. tests above 0.070.

The A.D.S.A. Score Card for Milk. Scoring of milk samples in this investigation was done in accordance with the score card formulated and approved by the American Dairy Science Association and used in most official and educational milk judging in the United States. A detailed description of the score card and the manner in which it is used has been published by Nelson and Trout *(10)*. Flavor is allowed 45 points out of a total of 100, but milk is never given a perfect flavor score. The normal range is from 25 to 40 points with the majority of commercial market milk scoring between 30 and 40. Any milk unfit for use as market milk is given a 0 score for flavor. In general, milk scored 40 points or above may be considered excellent; 37 to 40, good; 34 to 37, fair; 25 to 34, poor; and 0 to 25, bad. Criticisms identifying the predominant defect or defects are given for any samples scored below 40. Milk having a detectable oxidized flavor is scored 37 or below. Slightly oxidized milk is usually scored between 34 and 37, distinctly oxidized between 25 and 34, and pronounced oxidized below 25.

Harrison and Elder *(6)* noted the importance of providing key samples to fix standards for numerical scoring systems. In the judging of most dairy products uniform standards for flavor defects are not available and, consequently, reliance must be placed on the ability of trained tasters to standardize their scores without the use of flavor standards. Key samples were included in Experiment 1, but not in Experiments 2 and 3.

In the experiments described below, duplicate or triplicate portions of each milk were presented to the scorers in random order. Two criteria were used to evaluate the consistency of the scorers. The number of duplicate or tripliclate sets which were not criticized consistently with respect to oxidized flavor (either present or not) was used as an indication of the ability of the scorer to identify the defect. The standard deviation of duplicate or triplicate scores (adjusted as described below) was used as a measure of consistency in giving numerical scores. The deviations of the duplicate or triplicate scores from their means were used in calculating the standard deviations.

In using the A.D.S.A. score card, scorers tend to use half points above 38, whole points from 30 to 38, and much wider intervals below 30. There is some justification for this practice in Weber's law *(11)* which indicates that a geometrical increase in amount of flavored compound is necessary to produce an arithmetic increase in flavor intensity. For this reason a score card with graded intervals might be expected to correlate more directly with concentration of the flavored substance than uniform arithmetic scores. In determining the standard deviations of the scores, the varied scoring interval placed a severe handicap on the scorers who tended to score the samples more severely. For this reason, in analyzing the data for these experiments, scores below 30 were arbitrarily adjusted to compensate partially for the gradation in scoring intervals used. In practice the interval most frequently used below 30 is 5 points. In adjusting the scores this interval was arbitrarily reduced to 2 points, so that a score of 25 was adjusted to 28, score 20 to 26, 15 to 24, 10 to 22, and since no samples were scored between 0 and 10, score 0 was adjusted to 20. All flavor scores reported in this paper have been adjusted in this manner.

RESULTS AND DISCUSSION

Three experiments designed to determine the relation between results of the T.B.A. test and organoleptic flavor scores of milk having an oxidized flavor are described below.

Experiment 1. In the study by Trout and Sharp *(11)* of the reliability of flavor judgments with special reference to oxidized flavor, series of samples were ranked in increasing order of oxidized flavor. In Experiment 1 both ranking and numerical scoring were applied to the same series of samples to provide information regarding the reliability of flavor judgments by the two procedures. Fresh pasteurized milk and milk having an oxidized flavor catalyzed by the addition of 0.1 p.p.m. of copper were mixed in different proportions to give a series of nine samples with increasing intensities of oxidized flavor. Each sample was divided into six portions, four to be used for part A and two for part B.

In part A, four scorers who were familiar with oxidized flavor and the A.D.S.A. score card were given the nine samples under code numbers and in random order and were asked to rank them in increasing order of oxidized flavor. The temperature of the samples had been adjusted previously to approximately 20° C. The results are presented in Table 1.

The 18 portions for part B were arranged in random order, given code numbers, tested by the T.B.A. procedure and scored by the four scorers. Additional portions of Nos. 1 and 7 were submitted along with the coded series as key samples to fix flavor standards and were given scores and criticisms of 38 Feed and 30 Oxidized, respectively. Table 2 contains summarized results of Experiment 1B.

The fresh pasteurized milk used in this experiment had a feed flavor which may have masked oxidized flavor of low intensity. The presence of feed flavor

TABLE 1

Order of increasing oxidized flavor as ranked by four scorers in series of samples prepared by mixing normal and oxidized milk (Experiment 1A)

No.	Percent oxidized milk in sample	Order of increasing oxidized flavor by scorer				Ranking errors
		A	B	C	D	
1	0	1	1	1	1	0
2	10	2	2	3	2	1
3	20	3	3	2	4	2
4	30	4	5	4	3	2
5	40	5	4	5	5	1
6	50	7	7	6	7	3
7	60	6	8	7	8	3
8	80	8	6	9	6	5
9	100	9	9	8	9	1
Ranking errors		2	6	4	6	

complicates the interpretation of the scores for establishing a relation between oxidized flavor and results of the T.B.A. test and for comparing ranking and scoring as methods for estimating the intensity of oxidized flavor. Criticisms for feed flavor were given eight times for No. 1, three times for No. 2 and once each for Nos. 3 and 4, and the cuts in score probably were approximately in proportion to the number of criticisms noted. If feed flavor had not been present, No. 1 would probably have scored 40 and No. 2 between 37 and 40. In Tables 1 and 2 the ranking errors were calculated by allowing 1 point for each place by which the samples were out of order. Since No. 1 was not criticized for oxidized flavor, its score was considered to be 40 for calculating the ranking errors in Table 2.

The total number of ranking errors was approximately the same whether the samples were ranked in increasing order of oxidized flavor or given numerical scores (18 and 15, respectively). Direct comparison of these ranking errors may not be valid because the samples were included singly in part A but in duplicate in part B. However, the results indicate that there is little difference in the reliability of ranking and scoring as measures of oxidized flavor. For the purposes of this investigation, numerical scores were preferred because they provided more information regarding differences in intensity of oxidized flavor.

In applying statistical analysis to the data in Table 2, the scores for No. 1 were considered 40 since this sample did not receive any "oxidized" criticisms, and results for No. 2 were omitted because it was not possible to correct for the influence of feed flavor on the scores of this sample. The coefficients of correlation show that the T.B.A. tests correlated more closely with the percentage of oxidized milk than did the flavor scores.

Experiment 2. The samples used in this experiment were prepared by adding small amounts of copper sulfate solution to pasteurized milk and storing at 2° C. for two to five days. Fourteen samples with varied intensity of oxidized flavor were divided into three portions, one of which was used for T.B.A. tests (single determinations) and the others were coded with random numbers and scored by the same scorers as in Experiment 1. One of the scorers criticized two samples for slight feed flavor. The scores for these samples were arbitrarily raised from 39 to 40 to minimize interference of defects other than oxidized flavor. Table 3 contains the results of this experiment.

Results for two samples, Nos. 4 and 12, show considerable deviation from the general trend. In the case

TABLE 3

T.B.A. tests and flavor scores of milk samples in which oxidized flavor was induced by copper additions (Experiment 2)

No.	T.B.A. test (Optical density)	Average of duplicate flavor scores by scorer				Average of scores by 4 scorers	No. of "oxidized" criticisms
		A	B	C	D		
1	0.043	40	40	40	40	40.0	0
2	0.047	40	35.5	40	40	38.9	2
3	0.048	40	40	40	40	40.0	0
4	0.048	32.5	36.5	36.8	38.5	36.1	7
5	0.049	37	40	40	40	39.2	1
6	0.051	37	40	38.5	39	38.6	6
7	0.053	37.5	37	37.5	37.5	37.4	6
8	0.061	35	35.5	35	36	35.4	8
9	0.064	36	35.5	34	35.5	35.3	8
10	0.077	35	32	31.5	32.5	32.8	8
11	0.079	35	32	29	24	30.0	8
12	0.081	24.5	28	24	20	24.0	8
13	0.093	36	28	30.5	31.5	31.5	8
14	0.116	26	27	27	23	25.8	8
Standard deviation of duplicates		1.55	1.28	0.86	1.83	.....	.....
No. of inconsistent "oxidized" criticisms		3	1	1	1	.....	.....

TABLE 2

T.B.A. tests and flavor scores for series of samples prepared by mixing normal and oxidized milk (Experiment 1B)

No.	Percent oxidized milk in sample	Average of duplicate T.B.A. tests (Optical density)	Average of duplicate flavor scores by scorer				Average of scores by 4 scorers	No. of "oxidized" criticisms
			A	B	C	D		
1	0	0.040	37	34.5	38	38.5	37.0	0
2	10	0.044	38	35.5	37.5	39	37.5	3
3	20	0.051	36	35	37	37.2	36.5	7
4	30	0.056	37	35.5	37	37.5	36.8	7
5	40	0.062	29.5	30	35.5	34.5	32.4	8
6	50	0.072	31	33.5	34.5	33	33.0	8
7	60	0.079	28.5	29	30.5	28	29.0	8
8	80	0.080	29	28	29	28	28.5	8
9	100	0.098	28.5	28	29	28.5	28.5	8
Standard deviation of duplicates		0.0023	0.87	0.57	1.24	0.77	.....	.....
No. of inconsistent "oxidized" criticisms		.....	1	0	1	1	.....	.....
Ranking errors [b]		0	6	4	0	5	.....	.....
Coefficient of correlation with percent oxidized milk		0.98	—0.86	—0.90	—0.96	—0.93	—0.94	.....

[b] No. 1 considered to have score 40 since it was not criticized for oxidized flavor.

of No. 4, the deviation may be attributed principally to inconsistent scoring by one scorer. A's scores for the duplicate portions were 30 and 35, whereas the average for scorers B, C and D was 37.2. It is improbable that the flavor scores for No. 12 were in error enough to account for the observed deviation from the general trend because three of the four scorers gave this sample the lowest score. The T.B.A. test for this sample could have been in error. The coefficient of correlation between the T.B.A. tests and average scores by the four scorers is —0.68, but this value is increased to —0.94 if the results for No. 12 are omitted.

Experiment 3. The samples used in Experiments 1 and 2 were prepared samples in which the oxidized flavor was not necessarily representative of that which occurs in commercially processed milk. For Experiment 3, 20 samples were selected from commercially processed market milk exhibited at the 1950 California State Fair. These samples were five to seven days old at the time they were used in this experiment and several had at least two flavor defects. Five additional samples were included: fresh raw milk, fresh commercially pasteurized milk, fresh homogenized milk, and two pasteurized samples which had been exposed to daylight. The defects present and the approximate numbers of samples in which they occurred were oxidized, 14; feed, 6; cooked, 3; and light, 2.

Immediately before the samples were scored each was mixed thoroughly and four portions were transferred to half-pint bottles. One portion was used for T.B.A. tests but since no copper determinations were made, the results were not corrected for possible interference of copper. The three remaining portions were coded with random numbers from 1 to 75 and scored by seven individuals. Scorers A, B, C, and D were the same as in Experiments 1 and 2, E was also familiar with the A.D.S.A. score card, whereas F and G were relatively inexperienced in scoring milk but were familiar with the scoring of other products. The 75 samples were poured for the scorers at a rate of approximately one per minute and little opportunity was allowed for rechecking.

The results of the T.B.A. tests and averages of the triplicate flavor scores for each sample by the seven scorers are tabulated in Table 4.

Because of the limited experience of F and G in scoring milk, their results were not included in the statistical analysis of the data but are presented in the table for comparison with those of the more experienced scorers. In studying the relation between oxidized flavor and T.B.A. tests, greatest confidence may be given to those samples which were criticized most consistently as either oxidized or not oxidized because in some samples other defects may have obscured oxidized flavor of low intensity. For this reason only those samples which were criticized as oxidized less than four times or more than 11 times by scorers A, B, C, D, and E were included in the statistical analysis. The coefficient of correlation between the T.B.A. tests and average flavor scores for these samples is —0.95.

The results for Nos. 3 and 7 warrant special comment. The day before the experiment these samples were exposed to sunlight in quart bottles for 30 min. and 1 hr., respectively. Light produces a characteristic flavor defect which is sometimes confused with both cooked and oxidized flavors. Furthermore, light usually catalyzes the development of oxidized flavor. No. 3 was given seven cooked, four light, and four oxidized criticisms, and No. 7 was given seven cooked, six light, one oxidized, and one rancid criticism. The scorers were not as familiar with this defect as with other common flavors and obviously had difficulty identifying it. The

TABLE 4

T.B.A. tests and flavor scores of commercially processed milk samples (Experiment 3)

No.	T.B.A. test (Optical density)	Average of triplicate flavor scores by scorer							Average of flavor scores by A, B, C, D, E	No. of "oxidized" criticisms by A, B, C, D, E
		A	B	C	D	E	F	G		
1	0.035	39.3	40.0	39.0	39.2	39.3	36.0	39.7	39.4	1
2	0.039	39.0	40.0	38.2	40.0	38.7	38.0	40.0	39.2	0
3	0.053	39.0	38.0	35.0	36.2	37.0	33.0	38.5	37.0	4
4	0.058	37.0	38.0	37.8	38.8	40.0	38.7	38.3	38.3	6
5	0.058	37.7	39.7	37.8	38.7	36.5	37.3	38.7	38.1	3
6	0.060	37.0	38.3	38.3	36.2	39.0	34.7	39.3	37.8	6
7	0.064	39.0	37.0	33.3	36.7	34.3	35.8	37.7	36.1	1
8	0.066	35.3	37.3	38.0	35.5	37.0	37.7	37.0	36.6	7
9	0.068	37.3	38.0	36.2	39.2	39.0	37.8	38.0	37.9	4
10	0.068	35.3	37.0	36.8	38.3	37.5	32.0	35.7	37.0	8
11	0.071	37.3	39.0	37.5	38.0	39.3	34.3	38.0	38.2	7
12	0.071	36.7	37.7	35.8	38.8	39.3	35.0	39.0	37.7	4
13	0.072	35.0	36.3	36.0	36.7	37.8	36.0	36.7	36.4	11
14	0.077	35.0	33.7	32.7	35.7	34.5	35.2	35.3	34.3	15
15	0.084	37.0	36.7	35.0	36.7	37.8	35.7	35.3	36.6	12
16	0.093	34.7	34.7	31.7	35.8	36.0	33.3	32.0	34.6	15
17	0.095	34.7	36.0	34.0	36.0	38.3	35.7	38.0	35.8	13
18	0.095	30.0	34.0	32.7	35.0	36.7	32.8	33.2	33.7	14
19	0.098	34.3	37.3	35.7	39.2	38.3	36.8	37.5	37.0	9
20	0.098	33.3	36.0	36.5	33.7	38.0	36.7	38.5	35.5	14
21	0.098	34.3	34.0	32.3	33.3	37.0	31.3	36.7	34.2	15
22	0.107	31.3	32.7	29.3	33.0	35.8	33.8	35.0	32.4	15
23	0.114	32.0	35.3	28.7	33.3	37.5	30.7	34.7	33.4	14
24	0.142	34.0	32.3	30.7	35.0	34.0	31.0	32.7	33.2	14
25	0.164	31.3	31.3	28.0	30.7	28.7	33.3	31.3	30.0	15
Standard deviation of triplicates		1.34	1.57	1.53	1.38	1.48	2.63	2.22	.....	.....
No. of inconsistent "oxidized" criticisms		7	9	3	11	11	11	12	.....	.....

light flavor probably masked oxidized flavor of low intensity in these samples.

Relation Between Flavor Scores and T.B.A. Tests. Table 5 contains coefficients of correlation between average flavor scores and T.B.A. tests and regression lines calculated by the method of least squares for each experiment.

TABLE 5

Coefficients of correlation between flavor scores and T.B.A. tests and regression of T.B.A. tests on flavor scores

	Coefficient of correlation	Regression line [c]
Experiment 1B	—0.92	$y = 0.035 - 0.0045\ x'$
Experiment 2 [d]	—0.94	$y = 0.042 - 0.0048\ x'$
Experiment 3	—0.95	$y = 0.026 - 0.0134\ x'$

[c] x′ is x — 40 and x is average flavor scores.
y is optical density of T.B.A. test.
[d] Results for sample No. 12 omitted.

The coefficients of correlation indicate that there was a close inverse relation between the flavor scores and the T.B.A. tests in each of the experiments. The reliability of the flavor scores appears to be the principal factor limiting the concordance of the data since in Experiment 1B the coefficient of correlation between the percentage of oxidized milk in the samples and the T.B.A. tests was higher than the correlation coefficients between percentage of oxidized milk and flavor scores (Table 2).

The regression lines are presented in a form such that the x′-intercepts represent the estimated optical density for samples with a flavor score of 40. The lines for Experiments 1B and 2 show as close agreement as may be expected without a means of fixing flavor standards from day to day, but the slope of the regression line for Experiment 3 is considerably greater than for Experiments 1 and 2.

Neither the T.B.A. tests nor the flavor scores for Experiment 3 were considered as reliable as those for Experiments 1 and 2. Corrections for interference of copper in the T.B.A. test were made in Experiment 1B and 2, but not in Experiment 3. Consequently, the T.B.A. tests for Experiment 3 probably were too high, particularly those for the most oxidized milks. If copper corrections had been applied, the slope of the regression line would have been closer to that obtained in Experiments 1B and 2.

Several factors limited the reliability of the flavor scores in Experiment 3. The large number of samples scored in the relatively short time (75 samples in approximately 75 min.) may have caused enough fatigue to reduce the scorers' sensitivity. The samples were more difficult to score because several flavor defects were included in the series, and in some cases two or more defects were present in the same sample. Key samples were not provided as fixed flavor standards. Although the standard deviations of triplicate scores for the individual scorers were similar to those for the duplicate scores in Experiment 2, the slopes of the regression lines for the individual scorers varied more than in Experiments 1B and 2. For these reasons the relation between flavor scores and T.B.A. tests in Experiments 1B and 2 is considered more dependable than that in Experiment 3.

As a guide to aid in fixing standards for oxidized flavor in milk, on the basis of results of Experiments 1B and 2, samples with flavor scores of 37, 34, and 30 would be expected to have T.B.A. tests of approximately 0.050, 0.065, and 0.085, respectively.

SUMMARY

A method for the chemical estimation of oxidized flavor based on the formation of a red color when oxidized milk is acidified and heated with 2-thiobarbituric acid is outlined.

The relation between results of the thiobarbituric acid (T.B.A.) test and organoleptic flavor scores of milk samples having oxidized flavor was investigated. In an experiment in which a series of samples with varied intensity of oxidized flavor was prepared by mixing normal and oxidized milk in different proportions, the correlation between the T.B.A. test and percentage of oxidized milk in the samples was closer than that between the flavor scores (adjusted to compensate partially for graded scoring intervals) and the percentage of oxidized milk. A close correlation between results of the T.B.A. test and adjusted flavor scores was also found in an experiment in which oxidized flavor of varied intensity was catalyzed by the addition of copper, and in one involving commercially processed milk samples. Regression lines indicating the relation between adjusted flavor scores and the T.B.A. test are presented.

Acknowledgments

The author gratefully acknowledges valuable suggestions of G. A. Baker regarding the statistical treatment of the data, the generous cooperation of the scorers who participated in these experiments, and technical assistance of W. G. Jennings, H. O Smith, and M. Nury.

LITERATURE CITED

1. Abramson, H. The oxidation of unsaturated fatty acid in normal and scorbutic guinea pigs. *J. Biol. Chem.*, **178**, 179 (1949).
2. Bernheim, F., Bernheim, M. L. C., and Wilbur, K. M. The reaction between thiobarbituric acid and the oxidation products of certain lipides. *J. Biol. Chem.*, **174**, 257 (1948).
3. Bernheim, F., Wilbur, K. M., and Fitzgerald, D. B. Studies on a new metabolite and its oxidation in the presence of ascorbic acid. *J. Gen. Physiol.*, **31**, 195 (1947).
4. Brown, W. C., and Thurston, L. M. A review of oxidation in milk and milk products as related to flavor. *J. Dairy Sci.*, **23**, 629 (1940).
5. Greenbank, G. R. The oxidized flavor in milk and dairy products: a review. *J. Dairy Sci.*, **31**, 913 (1948).
6. Harrison, S., and Elder, L. W. Some applications of statistics to laboratory taste testing. *Food Technol.*, **4**, 434 (1950).
7. Kohn, H. I., and Liversedge, M. On a new aerobic metabolite whose production by brain is inhibited by apomorphine, emetine, ergotamine, epinephrine and menadione. *J. Pharmacol. Exptl. Therap.*, **82**, 292 (1944).
8. Kuck, S. K. D. Further studies on the factors which affect the lipide oxidation in rat liver as estimated by the thiobarbituric acid test. *Arch. Biochem.*, **26**, 351 (1950).
9. Lea, C. H. Rancidity in edible fats. Dept. Sci. Ind. Research, (Brit.) Food Invest. *Special Repts.*, **46** (1938).
10. Nelson, J. A., and Trout, G. M. *Judging Dairy Products*, 2nd Ed., 1948, Olsen Publishing Company, Milwaukee, Wis., 494 pp.
11. Trout, G. M., and Sharp, P. F. The reliability of flavor judgments with special reference to oxidized flavor of milk. N. Y. (Cornell) Agr. Expt. Sta. Memoir 204, 60 pp. (1937).
12. Wilbur, K. M., Bernheim, F., and Shapiro, O. W. The thiobarbituric acid reagent as a test for the oxidation of unsaturated fatty acids by various agents. *Arch. Biochem.*, **24**, 305 (1949).